

DETERMINING THE IMPACTS OF OCEAN ACIDIFICATION  
AND GLOBAL WARMING ON BIOTIC INTERACTIONS IN  
SHALLOW-WATER MARINE ENVIRONMENTS

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*I'll be more enthusiastic about encouraging thinking outside the box when there's evidence of any thinking going on inside it.*

— Terry Pratchett

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## ABSTRACT

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Ocean acidification (OA) and warming represent two of the greatest challenges of the 21<sup>st</sup> century, and understanding how they will influence whole ecosystems and their functionality is crucial for effective management. My goal was to determine how climate change affects species and community assemblages by investigating both direct (physiological responses), and indirect (species interactions) effects. In Chapter 2, a meta-analysis was presented that suggested there are 'winners' and 'losers' in climate change, alongside some predictable trait-based variation in sensitivity/resilience that can be attributed to taxonomic groups and/or life stages. Importantly, it was also demonstrated that the combination of OA and warming generally interacted synergistically. In the first experiment (Chapter 3), aquarium-based mesocosms (also used in Chapters 4 and 5) were used to demonstrate that OA and warming not only directly alter species (individual) physiological performance, but also their predator-prey dynamics due to the reduction of prey quality. It was found that the reduced prey quality was sufficient enough to prevent the predator from being able to initiate compensatory feeding, and they subsequently exhibited tissue loss. This highlights the need to simultaneously consider the inclusion of those species that are trophically linked. In Chapter 4, the role of the predator in eliciting non-consumptive effects (i.e. foraging-refuge trade-off) was additionally considered. The results suggest that future ocean acidification and warming govern the diminishing energy budget of consumers, which requires them to be less risk adverse, but ultimately more susceptible to predation themselves. As a consequence, the strength of non-consumptive effects appear to reduce as energy reserves are exhausted, and the behavioural choices of the organisms become driven instead by the need to reduce starvation risk. In Chapter 5, the effects of OA and warming on a biodiversity-ecosystem functioning relationship were tested. In general, it was found that increased biodiversity appeared to either promote or at least sustain ecosystem multi-functionality (i.e. the positive functioning of the ecosystem, across several ecosystem processes). However, when the temperature was elevated it was no longer possible to sustain this multi-functionality due to a shift towards heterotrophy, resulting in trade-offs between ecosystem processes. In Chapter 6, the longer-term consequence of OA were considered, and an *in-situ* CO<sub>2</sub> seep site was used to empirically link the energetic consequences of the individual to the contemporary demographic processes of the population. The results suggest that ocean acidification is driving individual and population level changes that will alter eco-evolutionary trajectories. Moreover, the results also suggest that the altered population demographics will lead to greater levels of short-term genetic drift that is predicted to oppose adaptation. Overall, these results illustrate how the effects of future OA and warming are likely to have important individual-, population-, community- and ecosystem-level consequences, with further interactions between these levels of biological hierarchy. Achieving a more holistic understanding of these responses is imperative for the adaptation and management of climate change.



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## PREFACE

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Some of the work included in this dissertation has been published or submitted for publication to peer-reviewed scientific journals, and therefore some contributions were made by collaborators. Subsequently, this thesis is written at times in the first-person, plural personal pronoun (i.e. we, our).

Chapter 2, I collected and analysed the data, and wrote the first manuscript draft. Dylan Gwynn-Jones and Pippa Moore contributed text and editing to the manuscript as supervisory authors. The paper was published as follows:

Ben P. Harvey, Dylan Gwynn-Jones and Pippa J. Moore (2013) Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecology and Evolution*, 4: 1016-1030.

Chapter 3, I designed the experiment, conducted all experimental work and data analyses, and wrote the manuscript. Pippa Moore was the supervisory author on this project and involved throughout the project in concept formation, manuscript edits and discussions. The manuscript was submitted as follows:

Ben P. Harvey and Pippa J. Moore (submitted) Ocean warming and acidification prevents compensatory response in a predator to reduced prey quality. *Oikos*.

For Chapter 4, I designed the experiment, conducted all experimental work, data analysis, and wrote the chapter. Pippa Moore was the supervisory author on this project and involved throughout the project in concept formation, manuscript edits and discussion.

For Chapter 5, I designed the experiment, conducted all experimental work (with assistance from Nathan King in conducting PAM Fluorometry), analysed all data, and wrote the chapter. Advice on experimental design and interpretation was provided by John Griffin (Swansea University). Pippa Moore was the supervisory author on this project and was involved throughout the project in concept formation, manuscript edits and discussion.

Chapter 6, the study was designed in collaboration with Pippa Moore. Fieldwork was performed by myself and Pippa Moore, with support from Andy Foggo. Organism respirometry was carried out by Samuel Rastrick with support from Camilla Bertolini, Helen Graham and Daniel Small. Molecular work was carried out by myself, Niall McKeown and Paul Shaw. Seawater carbonate chemistry was analysed by Samuel Rastrick, Jason Hall-Spencer and Marco Milazzo. I wrote the first manuscript draft, with editing and discussions principally from Niall McKeown, Samuel Rastrick, Andy Foggo, Paul Shaw, Daniel Small, and Pippa Moore. This manuscript has been submitted as:

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Part I

MAIN THESIS



## GENERAL INTRODUCTION

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## 1.1 INTRODUCTION

The concentration of atmospheric carbon dioxide (CO<sub>2</sub>), that had remained in the range of 172-300 parts per million (ppm) over the past 800,000 years (Lüthi *et al.*, 2008), has been steadily increasing since the industrial revolution to reach 391 ppm by the end of 2011 (Le Quéré *et al.*, 2012). The oceans and the terrestrial biosphere act as a considerable sink for this atmospheric CO<sub>2</sub>, with the surface waters of the oceans estimated to have absorbed about 25 % of CO<sub>2</sub> since 1800 (Sabine *et al.*, 2004). The persistence of atmospheric CO<sub>2</sub> increases natural greenhouse effects, and causes changes in the climate (IPCC, 2013). Over the past century, global sea surface temperatures have increased by 0.74 °C (Trenberth *et al.*, 2007), while the oceans have also shown a reduction of 0.1 pH units from pH 8.2 to pH 8.1 (Caldeira & Wickett, 2003; Kleypas *et al.*, 2006) due to changes in ocean carbonate chemistry, termed ocean acidification (Orr *et al.*, 2005).

Future levels of atmospheric CO<sub>2</sub> are highly dependent on global socio-economic responses, however, using estimates for a worst-case scenario, CO<sub>2</sub> levels are predicted to reach approximately 1250 ppm by 2100 (Figure 1.1; RCP 8.5; IPCC, 2013). This is expected to result in global sea surface temperatures rising by a further 1-4 °C (Trenberth *et al.*, 2007), while the increase in dissolved carbon dioxide (CO<sub>2</sub> [aq]) will fundamentally alter the carbonate chemistry of oceans, resulting in the decreased availability of carbonate ions, with a further reduction in pH by 0.3-0.5 units (IPCC, 2013).

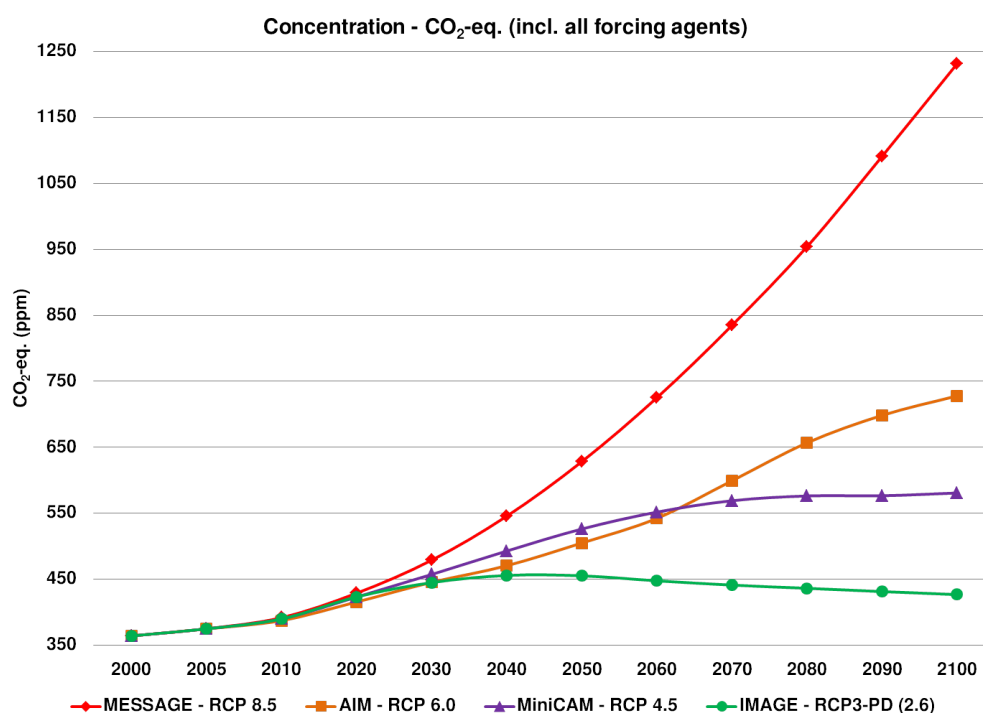


Figure 1.1: IPCC [2013] predictions for atmospheric CO<sub>2</sub> for the period 2000 to 2100. The representative concentration pathways (RCPs) are based on selected scenarios from four models, which integrated climate modelling, assessment modelling, and the analysis of impacts.

Ocean acidification and global warming are considered two of the greatest threats to marine ecosystems (Orr *et al.*, 2005; Hoegh-Guldberg & Bruno, 2010), yet, our understanding of the biological and ecological consequences of these prevalent abiotic drivers on marine organisms is limited (Orr *et al.*, 2009). As such, in recent years there has been an emergence of studies investigating the relationship between temperature and ocean acidification, and specifically how these stressors will interact to impact marine biota (see Harvey *et al.*, 2013; Kroeker *et al.*, 2013b for reviews). This is because ocean acidification and warming act in a mechanistically different manner, and yet are both influencing future populations at the same time. Recent meta-analyses across ecological systems (Crain *et al.*, 2008; Darling & Côté, 2008; Tylianakis *et al.*, 2008) have demonstrated that the impact of multiple stressors<sup>1</sup> may lead to effects that are less (antagonistic) or greater (synergistic) than expected from the individual stressors (Folt *et al.*, 1999). Such unpredictable effects are of particular concern because the response of an organism may also be modified further with competitive (e.g. Diaz-Pulido *et al.*, 2011) or trophic interactions (e.g. O'Connor, 2009; Russell *et al.*, 2013; Sanford *et al.*, 2014), reducing our ability to predict whether future marine communities will resemble those of today (Munday *et al.*, 2013).

The following chapter provides supportive evidence for the need to study the impacts of ocean acidification and ocean warming on the physiology and ecology of marine organisms, and the processes that structure marine ecosystems, including population demographics (e.g. gene flow, population size), and biotic interactions (e.g. predator-prey, plant-herbivore, multi-trophic interactions). More specifically, this chapter will outline: the biogeochemical effects of ocean acidification and warming (section 1.2); the biological consequences of these drivers, including the physiological mechanisms (section 1.3), how population and community-level processes will be affected (section 1.4), and the implications of these drivers in terms of biodiversity-ecosystem functioning (section 1.5). Finally, the aims and objectives of this thesis will be presented (section 1.6).

## 1.2 BIOGEOCHEMICAL EFFECTS OF OCEAN ACIDIFICATION AND WARMING

Ocean acidification refers to the reduction in the pH of the ocean, primarily associated by the uptake of atmospheric CO<sub>2</sub>, which alters fundamental acid-base balances and the chemical speciation of dissolved inorganic carbons (Doney *et al.*, 2009; Gattuso & Hansson, 2011). Ocean acidification comprises a series of dynamic, chemical reactions (see Figure 1.2) which can be described as follows.

Carbon dioxide is exchanged between the atmosphere and ocean by an equilibrium of atmospheric carbon dioxide (CO<sub>2</sub> (g)) and dissolved carbon dioxide [CO<sub>2</sub>] (equation 1.1), which are related by Henry's law in thermodynamics equilibrium.

$$\text{CO}_2 (\text{g}) = [\text{CO}_2]; K_0 \quad (1.1)$$

Where, [CO<sub>2</sub>] represents the sum of [CO<sub>2</sub> (aq)] and carbonic acid (H<sub>2</sub>CO<sub>3</sub>), and K<sub>0</sub> is

<sup>1</sup> What is a stressor? In this thesis, the drivers (environmental variables such as pH and temperature) that have been altered by human activities, will be often be referred to as "stressors" and will be used to describe the situation where the drivers have been altered to the extent that the likelihood of eliciting negative responses in physiological or ecological processes is increased (*sensu* Breitburg *et al.*, 2015).

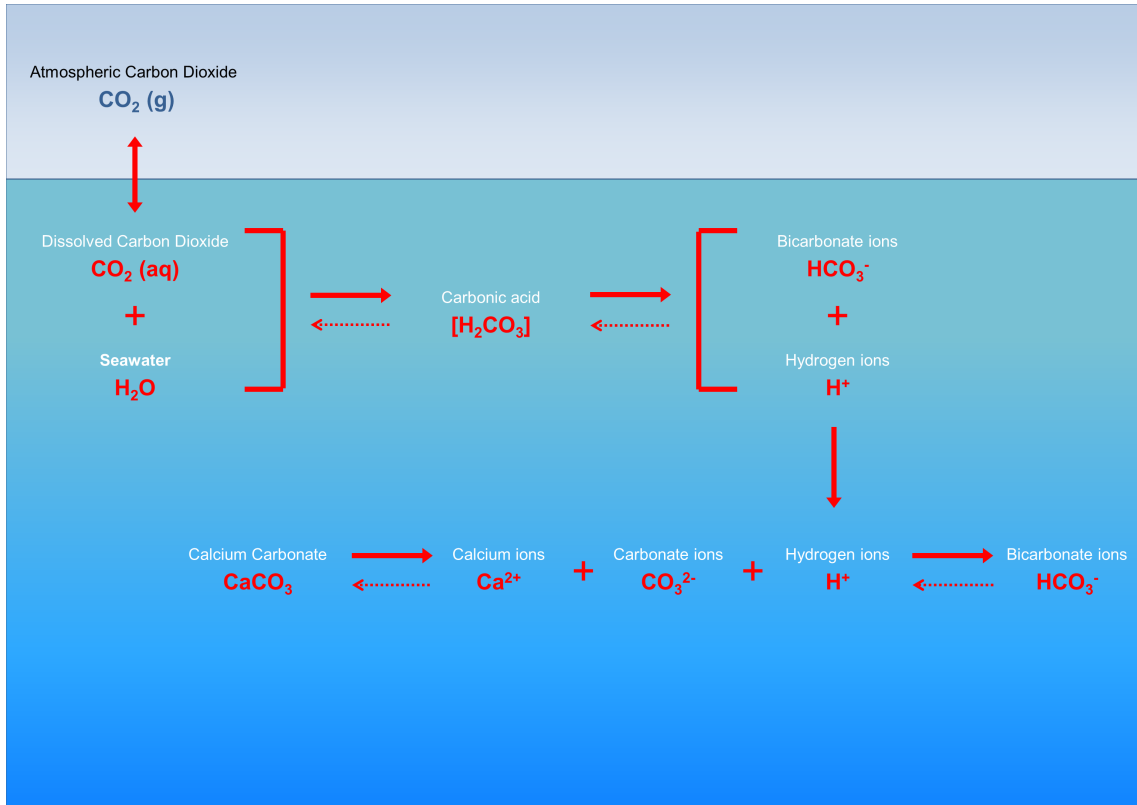
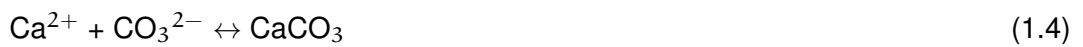


Figure 1.2: Schematic illustration describing the fate of atmospheric carbon dioxide into the marine environment. See text for more details.

the solubility coefficient of  $\text{CO}_2$  in seawater. Subsequently, the  $[\text{CO}_2]$  dissociates into bicarbonate ions ( $\text{HCO}_3^-$ , equation 1.2), which further dissociates into carbonate ions ( $\text{CO}_3^{2-}$ , equation 1.3).



Where  $K_1^*$  and  $K_2^*$  represent the stoichiometric dissociation constants of  $\text{H}_2\text{CO}_3$  (see [Lueker et al., 2000](#)), and  $\text{H}^+$  represents the concentration of protons (hydrogen ions). As  $\text{H}^+$  increases, the pH decreases. Dissolved inorganic carbon ( $\text{C}_\text{T}$ ) represents the sum of  $[\text{CO}_2]$ ,  $[\text{HCO}_3^-]$  and  $[\text{CO}_3^{2-}]$ , and as well as ocean acidification increasing the concentration of  $\text{C}_\text{T}$ , the speciation (proportions) between  $[\text{CO}_2]$ ,  $[\text{HCO}_3^-]$  and  $[\text{CO}_3^{2-}]$  will increase, while  $[\text{CO}_3^{2-}]$  will decrease (see Table 1.1). Since the precipitation of calcium carbonate  $[\text{CaCO}_3]$  (i.e. calcification) is controlled by the concentration of  $[\text{CO}_3^{2-}]$  (Equation 1.4), calcification rates are expected to decline with increasing ocean acidification.



Oceanic surface waters are naturally super-saturated with calcium carbonate, however the reduced availability of carbonate ions, associated with ocean acidification, increases the solubility of the three main biogenic  $\text{CaCO}_3$  minerals: aragonite, calcite, and magnesian calcite (mg-calcite). The  $\text{CaCO}_3$  saturation state ( $\Omega$ ) describes the solubility of

Table 1.1: Average changes in the carbonate chemistry of surface seawater from 2010 to 2100, for the AR4 SRES A2 and A1F1 scenario, and the AR5 RCP 8.5 scenario. Total Alkalinity ( $A_T$ ),  $pCO_2$ , temperature and salinity were used to calculate other parameters, using the software program, CO2SYS (Lewis & Wallace, 1998), with dissociation constants from Mehrbach *et al.* [1973], as adjusted by Dickson & Millero [1987], and  $KSO_4$  using Dickson [1990].

	2011	2100	2100	2100
Parameter	Present Day	A2	A1F1	RCP 8.5
pH (total scale)	8.072	7.826	7.715	7.62
Temp (°C)	18.9	22.4	22.9	24.5
Salinity (psu)	34.9	34.7	34.7	34.7
$A_T$ ( $\mu\text{mol kg}^{-1}$ )	2325	2310	2310	2310
$pCO_2$ ( $\mu\text{atm}$ )	391	750	1000	1300
$C_T$ ( $\mu\text{mol kg}^{-1}$ )	2058	2140	2183	2210
$HCO_3^-$ ( $\mu\text{mol kg}^{-1}$ )	1852	1983	2044	2079
$CO_3^{2-}$ ( $\mu\text{mol kg}^{-1}$ )	193	134	109	95
$CO_2$	13	23	30	37
$\Omega_{\text{calcite}}$	4.61	3.21	2.62	2.281
$\Omega_{\text{aragonite}}$	2.99	2.1	1.72	1.501

these minerals (Equation 1.5) that are utilised by marine organisms as skeleton and shell material. Aragonite is more soluble than calcite (150%), while the solubility of mg-calcite is dependent on the mole fraction of magnesium, with higher mole fractions being more soluble (Dickson, 2010).

$$\Omega = [Ca^{2+}][CO_3^{2-}] / K_{sp}^* \quad (1.5)$$

$$K_{sp}^* = [Ca^{2+}]_{sat}[CO_3^{2-}]_{sat} \quad (1.6)$$

Where,  $K_{sp}^*$  represents the solubility product for calcite or aragonite (Equation 1.6). When seawater is in equilibrium  $\Omega = 1$ , undersaturated when  $\Omega < 1$  (promoting inorganic dissolution), and supersaturated when  $\Omega > 1$  (promoting inorganic precipitation).



Pre-industrial pH levels remained relatively constant for 25 million years due to a process termed 'carbonate buffering' (Equation 1.7), which utilises the carbonate ions (present in the surface waters) to react with excess hydrogen ions and form bicarbonate ions (Doney, 2006; Widdicombe & Spicer, 2008). However, since this relies on the availability of carbonate ions (which are derived through relatively slow geological processes), the carbonate buffering system is now failing (Widdicombe & Spicer, 2008).



In addition to affecting the seawater carbonate chemistry system, atmospheric carbon dioxide is the principal anthropogenic greenhouse gas responsible for the process termed the greenhouse effect (Schneider, 1989). The increase of greenhouse gases in the atmosphere results in trapped thermal radiation, which causes a rapid increase in global temperatures (IPCC, 2007a, 2013). Over the last 100 years mean global temperatures have increased by  $0.74\text{ }^{\circ}\text{C} \pm 0.18\text{ }^{\circ}\text{C}$  (Trenberth *et al.*, 2007), with sea surface temperatures expected to continue increasing at a rapid rate and increase by  $5.6\text{ }^{\circ}\text{C}$  by the end of the century (IPCC, 2013). The median rate of warming is more than three times faster on land than at sea (Burrows *et al.*, 2011). This temperature difference between land and sea will therefore also affect the atmospheric circulation patterns, leading to more frequent and intense storms (Easterling *et al.*, 2000). One major concern, however, is that even should mitigation measures be implemented fully (and immediately), it will not succeed in keeping global temperature rises below  $2\text{ }^{\circ}\text{C}$  by 2100 (Kriegler *et al.*, 2013).

Ocean warming will cause alterations in the physical and chemical characteristics of seawater, which will additionally impact marine biota. Warming of surface waters may result in thermo-stratification and reduce nutrient upwelling, causing a decline in photosynthetic performance (Keeling & Garcia, 2002). Furthermore, increases in temperature will actually decrease the solubility of calcium carbonate, potentially ameliorating some of the deleterious effects of ocean acidification on the carbonate chemistry of seawater. However, the biological effects of combined acidification and warming on marine organisms remains largely unclear (Sala *et al.*, 2000; Fabry, 2008). Therefore the following sections will outline the biological and physiological effects that ocean acidification and warming have on marine biota, both independently and combined.

### 1.3 RESPONSES OF MARINE ORGANISMS TO ELEVATED $p\text{CO}_2$

Ocean warming and ocean acidification are recognised as pervasive and detrimental anthropogenic influences on marine life (Halpern *et al.*, 2008b). Despite acting in a mechanistically different manner, both drivers stand to alter behavioural and physiological traits, including the development and reproduction of marine organisms (Harrington *et al.*, 1999; Tylanakis *et al.*, 2008; Gilman *et al.*, 2010; Traill *et al.*, 2010; Kordas *et al.*, 2011), with the potential to scale up to population- and ecosystem-level impacts (Pörtner, 2002; Somero, 2005; Widdicombe & Spicer, 2008; Somero, 2010; Van der Putten *et al.*, 2010). The modern distribution of a species is shaped by a species' physiological limits and biotic interactions with co-existing species, both of which will play a key role in their response to future climate change (Somero, 2012). Marine organisms are typically considered to respond to a changing environment in one of three (non-exclusive) ways: spatially, temporally, and phenotypically (Bellard *et al.*, 2012).

Spatial responses include biogeographic range shifts such that organism responses are expected to find refuge through migration (e.g. Parmesan & Yohe, 2003; Root *et al.*, 2003; Poloczanska *et al.*, 2013). This is (predominantly) related to the tracking of isotherms in order to maintain their thermal niches. It is thought that such responses will be less associated with ocean acidification because spatial gradients in  $p\text{CO}_2$  are less common or structured in comparison to the predicted rate of (temporal) change (Kelly & Hofmann, 2013). Temporal responses highlight changes in phenological timing, such as reproduction and time of peak abundance (Parmesan & Yohe, 2003; Parmesan, 2006; Burrows *et al.*, 2011).

It is unlikely, however, that migration alone will be sufficient, forcing many species to adapt in order to survive (Shaw & Etterson, 2012). The third key response is through alterations in their physiology (or behaviour), while maintaining the same spatial and temporal structure that allows the organisms to persist (Bellard *et al.*, 2012). The relative importance of these mechanisms (i.e. spatial, temporal, and phenotypic responses) in tolerating environmental change will largely depend on the life-history characteristics of the organism, the rate and magnitude of the environmental change, the availability of suitable habitats (assuming a range shift) and the timescale over which climate change occurs (Holt, 1990; Davis & Shaw, 2001; Meyers & Bull, 2002).

Assessing the sensitivity of marine organisms to ocean acidification and warming requires that we first understand what traits are likely to be affected, and whether these particular traits can be generalised across different life-history characteristics and species/taxa. Physiological studies on extant species have suggested a number of convergent hypotheses to explain differences in the sensitivity of marine organisms to ocean acidification and warming. Although there are still species-specific responses, there is a suggestion of predictable trait-based variation in sensitivity that can be attributed to taxonomic groups or life stages (Fabry, 2008; Kroeker *et al.*, 2013b; Wittmann & Pörtner, 2013). For example, calcifying organisms are generally considered to be more sensitive to ocean acidification than non-calcifying organisms (Kroeker *et al.*, 2010). However, within that grouping, corals, echinoderms and molluscs are considered to be more sensitive to future (predicted) levels of  $p\text{CO}_2$  compared to (for instance) crustaceans (Wittmann & Pörtner, 2013).

### 1.3.1 *Calcification, acid-base regulation and metabolism*

For clarity, these physiological consequences will first be described in terms of ocean acidification and subsequently, ocean warming and (where applicable) any interaction with ocean acidification.

#### **Ocean acidification**

Calcification has received the most research attention of all the physiological processes likely to be influenced by ocean acidification because of the association with  $\text{C}_\text{T}$  (Kelly & Hofmann, 2013). Calcification can be thought of as the relative contributions in gross calcification and net dissolution rates. The threshold for the saturation states ( $\Omega_{\text{calcite}}$  and  $\Omega_{\text{aragonite}}$ ) that supports biogenic calcification is expected to be exceeded in the near future (Caldeira & Wickett, 2003; due to  $\text{CO}_3^{2-}$  decreasing as  $[\text{CO}_2]$  increases, see section 1.2). This means that the future ocean may become under-saturated with regards to the availability of calcium carbonate, and once  $\Omega < 1$ , will promote inorganic dissolution of the respective mineral.

However, while an extremely useful proxy, the carbonate saturation states do not directly set gross calcification rates *per se* (Pörtner, 2008; Hofmann *et al.*, 2010). This is because the site of calcification is (typically) spatially isolated from the seawater (Allemand *et al.*, 2004), and likely chemically different (Hofmann *et al.*, 2010). Moreover, the carbonate precipitated in the calcium structure does not in fact originate from the external seawater, instead it is generated or modulated from imported bicarbonate and/or  $\text{CO}_2$

within the alkaline compartment at the site of calcification (Allemand *et al.*, 2004; Carré *et al.*, 2006; Pörtner, 2008; Hofmann *et al.*, 2010). The saturation state of the seawater is instead predominantly influencing calcification by indirectly affecting the calcium and proton equivalent ion transport at the outermost barriers (e.g. gills, Pörtner, 2008), resulting in an increased energetic cost for active ion transport (Allemand *et al.*, 2004).

Changes in the physiochemical properties of seawater associated with climate change will result in  $\text{CO}_2$  being diffused across the outermost barriers (e.g. epithelia or equivalent) and affecting the extracellular acid-base status, with the extracellular pH ( $\text{pH}_e$ ) undergoing acidosis (i.e. decreasing). The weak acid distribution of  $\text{CO}_2$  means that each intracellular (membrane bound) compartment will have different pH ( $\text{pH}_i$ ),  $[\text{HCO}_3^-]$  and  $[\text{CO}_3^{2-}]$  values, due to their differential acid-base regulation (Pörtner, 2012).

In order to compensate for the extracellular acidosis occurring,  $[\text{HCO}_3^-]$  is accumulated in the extracellular (and sometimes intracellular) compartments, with any compensation of intracellular acidosis being further supported by transmembrane ion exchange back into the extracellular (Pörtner *et al.*, 1998). This process has been reported in the sipunculid worm *Sipunculus nudus* (L., 1766), which rapidly restored intracellular acidosis at the expense of a non-respiratory increase in the extracellular acidosis (Pörtner *et al.*, 1998). As a longer-term compensatory mechanism, complete non-bicarbonate compensation of  $\text{pH}_e$  may also be possible through increases in protein content (e.g. Velvet swimming crab, *Necora puber* (L., 1767), Small *et al.*, 2010; burrowing shrimp, *Upogebia deltaura* (Leach, 1815), Donohue *et al.*, 2012), with  $[\text{HCO}_3^-]$  buffering typically only used as a shorter-term compensation, for instance, in crustaceans (Whiteley, 1999, 2011). The implementation of such longer-term compensatory mechanisms should enable organisms to persist in future acidified oceans, albeit with an increased energetic cost.

The compensation for ocean acidification-induced changes in  $\text{pH}_e$  is energetically expensive, requiring ion pumps to drive the pH and ion regulation (Pörtner, 2008). During moderate stress from ocean acidification, this may cause a shift in the energy budget of cells, tissues or the whole organism (Melzner *et al.*, 2009; Lannig *et al.*, 2010; Sokolova *et al.*, 2012). This can include resources being reallocated from anabolic processes (e.g. protein biosynthesis) to vital processes for acid-base homeostasis (e.g. ion regulation; Sokolova *et al.*, 2012), in a process termed metabolic depression (see Guppy & Withers, 2007). While this will ensure short-term survival, it is not necessarily feasible in the longer-term, and could lead to reduced fitness in an organism (such as reduced growth rates or fecundity; Seebacher & Franklin, 2012).

It has been argued that the energetic costs of the proton pumps may be the key factor controlling calcification (Ries, 2011). As such, the sensitivity of (most) calcifying species (e.g. coral reefs; Anthony *et al.*, 2008) is likely not explicitly associated with their calcified structure. Instead, the sensitivity is associated with their incomplete compensation for disturbances in the extra- and intercellular acid-base status, as well as incurring the additional energetic costs associated with calcification itself (e.g. the active transportation of  $\text{HCO}_3^-$  to the site of calcification). Therefore, those species with a heavy calcified structure and lower capacity to adjust their internal pH (corals, echinoderms and molluscs) will be less tolerant compared to species that demonstrate high functional capacities of activity and associated metabolic pathways (including the capacity to regulate pH), and/or a less expressed calcified structure (crustaceans and fishes) (Pörtner, 2008; Melzner *et al.*,

2009; Pörtner, 2012; Wittmann & Pörtner, 2013).

### Ocean warming and interactions

Elevated temperatures in addition to ocean acidification will also play a key role in the physiological responses associated with acid-base disturbances, calcification mechanisms and other physiological processes. In general, elevated temperatures will speed up the kinetics of biological processes (Campbell & Farrell, 2009; Kordas *et al.*, 2011). For enzyme-catalysed reactions, increased temperatures will enhance the speed and efficiency of biochemical reactions by lowering the activation energy of a reaction, and allowing the substrate and enzyme to attain the transition state (Campbell & Farrell, 2009). However, eventually a temperature limit is reached, at which point the enzyme becomes denatured (with additional heat further denaturing the enzyme), rapidly slowing the reaction.

As a direct consequence, warming is expected to exponentially increase the metabolic rate of ectothermic organisms, leading to a kinetic stimulation of performance rates (Brown *et al.*, 2004), assuming this is within an organism's thermal tolerance range (Pörtner, 2012). Warming from lower temperatures towards an organism's optimum could support their capacity for ion exchange, including their ability to compensate for acid-base disturbances (Pörtner, 2012). Warming that exceeds their thermal tolerance, will reduce the aerobic scope for activity (Walther *et al.*, 2009), resulting in reduced energy availability (Pörtner & Knust, 2007). This is because for heterotrophic marine organisms, any increases in metabolic rate will also raise an organism's oxygen demand (Pörtner, 2010), unless acclimation or behavioural changes can compensate for respiratory loss (Sunday *et al.*, 2014).

The response of marine organisms to warming (as well as ocean acidification), is associated with an organism's capacity to meet their oxygen demand (see Figure 1.3). If the mechanisms that control oxygen supply (e.g. ventilation, circulation) become limited, such as metabolic depression reducing the rate of gas exchange across respiratory epithelia (Pörtner *et al.*, 2005), then internal oxygen stores will become depleted (hypoxaemia), and respiratory CO<sub>2</sub> will also typically accumulate. In order to deal with these adverse conditions, animals will go into metabolic depression and utilise anaerobic metabolism as a form of passive tolerance. This is commonly observed in the intertidal (e.g. due to oxygen deficiency or emersion from sea water). It is however, only utilised as a short-term mechanism to enable survival, it will not allow activities such as foraging, or performances like growth and reproduction and hence, is not feasible in the long-term (Pörtner, 2010, 2012). It must be noted that this loss in oxygen is not associated with the reduced concentration of ambient oxygen within the external seawater, rather by the inability to meet the internal temperature-dependent oxygen demands (Pörtner, 2010, 2012).

The effect of temperature on acid-base status and calcification is strongly dependent on whether the shift in environmental conditions will take the organism out of their optimal conditions (see Figure 1.3). It is possible that warming could potentially benefit the organism if oxygen supply can meet demand, particularly since temperature has additional positive effects on net calcification rates by decreasing the solubility of calcium carbonate, ameliorating the negative impacts of acidification (e.g. McNeil *et al.*, 2004; Kleypas & Yates, 2009). Importantly however, extreme temperatures that exceed optimal conditions

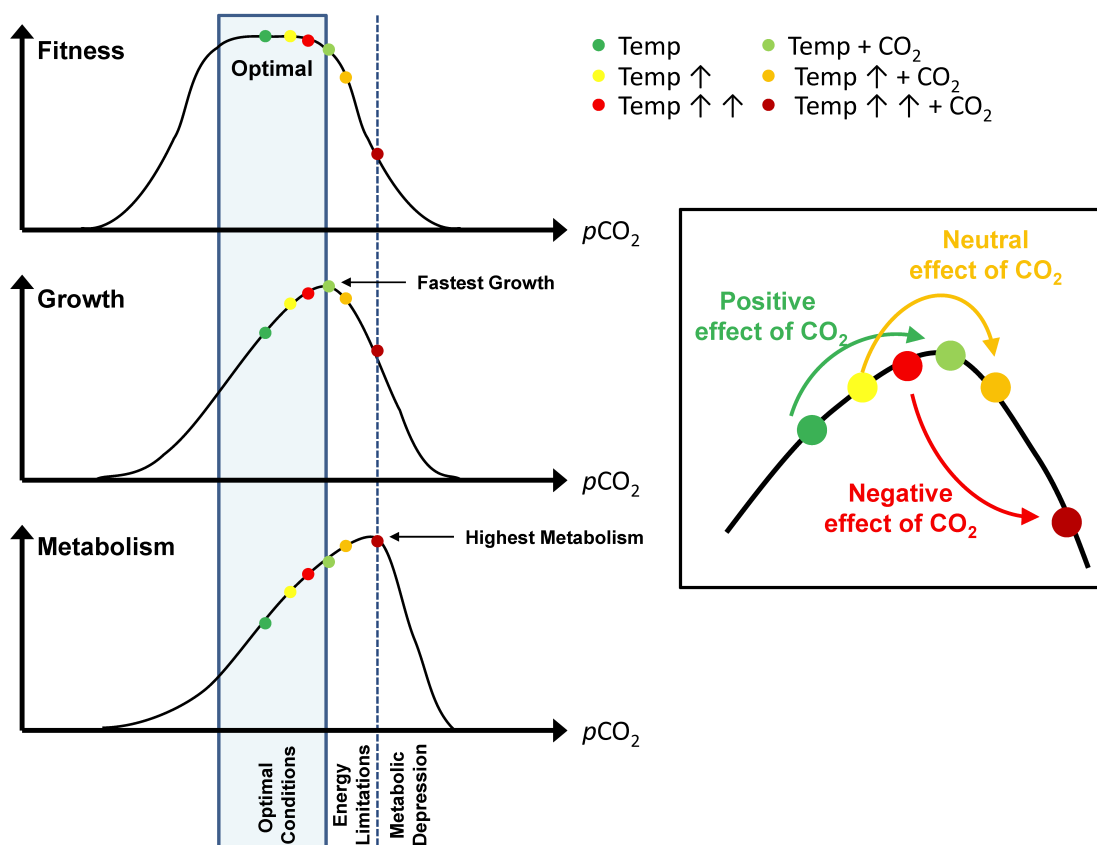


Figure 1.3: Theoretical model showing the link between fitness, growth and metabolism under ocean acidification and warming. Optimal conditions are defined as the extent of metabolism and growth that does not incur negative effects for fitness (i.e. to the point of energy limitation). Therefore, ocean acidification can lead to positive effects (not energy-limited, green dots), neutral effects (some energy limitation, yellow/orange dots) and negative effects (energy limited conditions, red dots) on growth rates (inset on right). This response is dependent on the starting metabolic rate, which is dictated by temperature. (Reproduced from [Gianguzza \*et al.\*, 2014](#)).

can result in a decline in an organism's capacity for aerobic activity (aerobic scope) (e.g. [Munday \*et al.\*, 2009b](#)) with deleterious consequences.

Ocean acidification-induced reductions in aerobic scope can occur even within an organism's optimal range of thermal tolerance ([Pörtner & Farrell, 2008](#)). This means that the limitations in their aerobic scope will be more rapidly imposed when ocean acidification and warming are interacting, because it will further decrease the capacity of an organism to increase its rate of aerobic energy turnover, limiting their performance. Therefore, elevated levels of  $\text{CO}_2$  will likely narrow an organism's optimal thermal window ([Pörtner \*et al.\*, 2005](#); [Pörtner & Farrell, 2008](#); [Pörtner, 2010, 2012](#)).

### 1.3.2 Energy budgets

The responses of marine organisms to ocean acidification and warming can be simply considered in terms of energy allocation ([Kooijman, 2010](#)). In general, energy is partitioned between those functions required for basal maintenance (e.g. metabolic rate) and the remainder is allocated towards the performance of additional activities such as



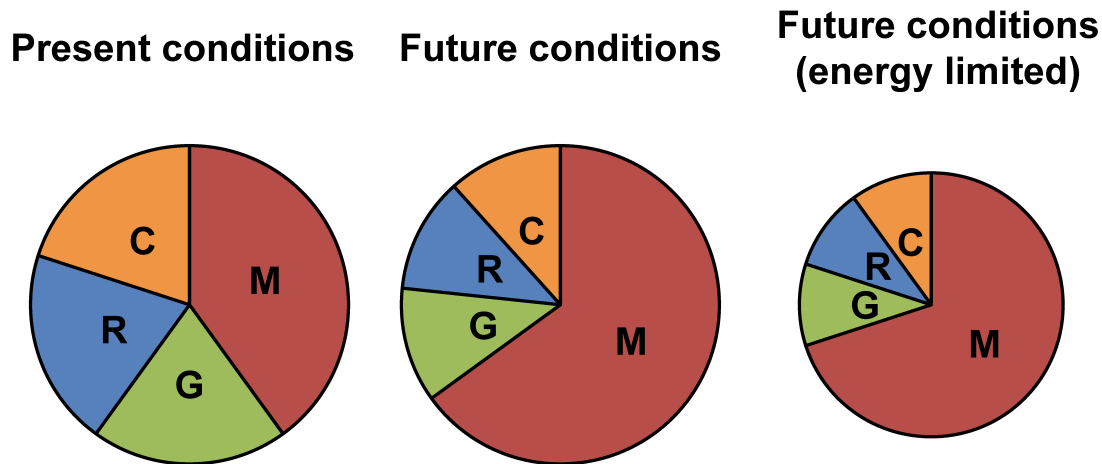


Figure 1.4: Schematic energy budget for an organism under present, future (with and without energy limitation) conditions. Under present conditions, maintenance (M) represents a substantial proportion of the total energy budget. Ocean acidification and warming could increase maintenance costs (e.g. acid-base homeostasis disturbances, or elevated respiration), resulting in less energy available for growth (G), reproduction (R) or calcification (C). Should future stressful conditions, also result in energy limitation, then the overall available energy will also be reduced (depicted by a smaller pie size) and result in even more energy (in relative terms) being allocated to maintenance, rather than growth, reproduction or calcification. Taken and modified from [Barry \*et al.\* \[2011\]](#).

stress resistance, biosynthesis (e.g. growth and reproduction), locomotion, foraging, digestion, and immune defences. Under stressful conditions, the allocation of energy towards maintenance costs could be increased (e.g. ion regulation, see section 1.3.1) and subsequently reduce the allocation of energy for the additional activities (Figure 1.4).

Should future conditions increase energy demand and insufficient energy be available (e.g. feeding rates not scaling with increased maintenance; discussed in detail in section 1.4.2), then trade-offs may occur between energy demanding processes (see Figure 1.4). For instance, the ophiuroid brittlestar, *Amphiura filiformis*, was capable of maintaining their calcification ability by increasing their metabolism. However, this energetic trade-off was achieved at the expense of muscle condition, suggesting that maintaining calcification is not a long-term compensatory mechanism ([Wood \*et al.\*, 2008](#)). Therefore, the ability of an organism to maintain elevated metabolic rates may allow them to sustain positive life-history traits (e.g. [Calosi \*et al.\*, 2013b](#)), and determine organism fitness in future acidified oceans (e.g. [Stumpp \*et al.\*, 2011](#)). Energy allocations will undoubtedly be species-specific, and yet a holistic view must be achieved to ensure that responses to climate change are not just viewed in terms of the performance of the individual (e.g. calcification rates). Instead, it is important to also consider how the adverse conditions will influence the fitness of the population (e.g. reproductive investment).

#### 1.4 POPULATION- AND COMMUNITY-LEVEL RESPONSES TO ELEVATED $p\text{CO}_2$

The target for much of climate change research is to establish explicit predictions regarding the population- and community-level effects of future climate change. Work associated

with global warming in particular, suggests that the majority of local population extinctions are not entirely associated with physiological responses to elevated temperature, instead primarily due to alterations in species interactions (Cahill *et al.*, 2013). Yet, the majority of climate change experiments to date have been relatively short-term, single species experiments (but see, for example, Miller *et al.*, 2012, 2013; Russell *et al.*, 2013; Palumbi *et al.*, 2014; Queirós *et al.*, 2014) and are therefore not easily extrapolated up to natural ecosystems.

In order to understand both longer-term, and community- and ecosystem-level responses requires multiple approaches. These include: (1) *in vivo* aquarium mesocosm experiments using multiple species or communities; (2) field observations using natural analogues, including both environmental gradients (e.g.  $\text{CO}_2$  venting or upwelling sites) and space-for-time manipulations (e.g. latitudinal temperature changes), as well as *in situ* mesocosm experiments (e.g. Free Ocean Carbon Dioxide Enrichment (FOCE) experiments); (3) long-term experiments which consider population demographics and examine the potential for acclimation (*sensu* Angilletta [2009]) and adaptation; (4) ecosystem modelling that can integrate the eco-physiological parameters of the individual with broad-scale biogeochemical simulations of environmental conditions and resource availability, and finally (5) combinations of the above. Examples utilising these approaches are subsequently discussed in further detail in the following sections: population demographics (section 1.4.1), and community interactions (section 1.4.2).

#### 1.4.1 Population demography

Despite evidence for the impacts of single stressors on individual species growing rapidly, research suggests that these climatic impacts will not necessarily translate directly into changes in distribution and abundance. Scaling up from individual to population-level responses will therefore require an appreciation of how climate change can not only influence the physiology of the individual, but also key demographic transitions that influence population dynamics.

Populations may be able to respond to climate change more rapidly through range shifts and phenotypic plasticity (acclimatisation), rather than from local adaptation (Merilá, 2012). However, the ecological processes and population demographics that control these different approaches will also themselves feedback and influence population regulation and persistence. For instance, many predictions of range shifts will use a species' current distribution to describe its environmental niche, and then use that niche in the context of future conditions (Pearson & Dawson, 2003; Sanford & Kelly, 2011). As such, the assumption is made that for a particular species, every population possess the same environmental niche. Should the populations have their tolerances dictated by phenotypic plasticity, then it is indeed possible that every population can persist in the full environmental niche. However, any local adaptation in a population means that different populations will have altered niches and some (e.g. species at their range edges) may be more susceptible to environmental change, and require gene flow from more tolerant populations to persist at their current range edge (Sanford & Kelly, 2011). An important component of population regulation and persistence is, therefore, population demographic rates and connectivity (i.e. the exchange of individuals among populations). For most benthic marine species with complex life cycles, population exchange will primarily occur during planktonic larval stages (Cowen & Sponaugle, 2009), but not exclusively.

## Life-history stages

Many organisms may be more vulnerable during early developmental and reproductive stages (Pörtner & Farrell, 2008; Dupont & Thorndyke, 2009; Ross *et al.*, 2011) compared to their adult life stages. This is particularly the case for individuals that calcify during their larval and juvenile phases, since many marine organisms will often begin the deposition of their shells and skeleton with an amorphous calcium carbonate, known to be  $\times 30$  times more soluble than the more stable forms of aragonite and calcite secreted later in their ontogeny (Orr *et al.*, 2005; Dupont *et al.*, 2010b).

Many marine species also have complex life histories, often going through numerous ontogenetic and ecological transitions during their development, each of which has different autonomy and sensitivity to environmental change (Dupont *et al.*, 2012). Phenotypic carry-over effects can occur between life-history stages (as well as trans-generationally, e.g. Miller *et al.*, 2012) that could exacerbate or alleviate the impacts on fitness-related traits (Podolsky & Moran, 2006). For example, larval experiences (such as due to food availability or exposure to stressful conditions) will not only impact juvenile performance, but could persist for the juvenile even when the adverse conditions are alleviated (e.g. Emlet & Sadro, 2006). This may be particularly important given that different life-stages may utilise different habitats or behaviour (e.g. Werner & Gilliam, 1984), and could therefore form a critical bottleneck effect should early life-history stages be disproportionately affected (e.g. during planktonic larval phase).

Different reproductive modes will also influence the response of marine organisms to environmental change. Broadcast spawning individuals, or those larvae with a planktonic stage may be strongly affected since the fertilisation of both eggs and sperm, and/or their development, will be occurring directly in the warmer acidified water (Ross *et al.*, 2011). Yet, the combination of ocean acidification and warming may lead to some opposing, sub-lethal responses. Ocean acidification has been demonstrated to cause prolonged larval development times, and reduced larval size (Dupont *et al.*, 2010c; Gazeau *et al.*, 2013), both of which may lead to an increase in the likelihood of predation during the larval phase (Rumrill, 1990). Antithetically, warming (within thermal tolerance limits) may stimulate metabolism in ectotherms and actually reduce development times, reducing the planktonic larval duration and hence, predation risk. It is therefore likely that since temperature-dependent effects in planktonic larval duration will attenuate with decreasing latitude, the effects of ocean acidification may be more prevalent in lower, warmer latitudes (Gaylord *et al.*, 2015). Thus, the vulnerability of early life-history stages to both ocean acidification and warming will have important effects on population demographics and survival, even when the adult life stages may be relatively unaffected.

### 1.4.2 Community interactions

The impact of climate change on marine communities will primarily be dependent on the different physiological susceptibility of interacting species, and requires understanding of how this will influence their performance in terms of their foraging, competition and community dynamics. The core assumption of any ecological interactions is that the consumers will likely be attempting to maximise their energy intake in comparison to their energy expenditure. Therefore, climate change represents an important shift since it is



capable of simultaneously influencing the resource availability as well as the energetic demand of the consumer.

### Competition and foraging

In competitive terms, marine autotrophs are likely to be competing for space rather than food, and many marine autotrophs are expected to benefit from the elevated levels of inorganic carbon in the future (Koch *et al.*, 2013; Brodie *et al.*, 2014), promoting growth and survival. Should ocean acidification or warming drive an enhanced inclination (or ability) to compete in one species, but not the other species, then it may influence patterns of dominance (McCormick *et al.*, 2013), such as enhanced space competition in macroalgae over corals (e.g. Diaz-Pulido *et al.*, 2011), or alternatively induce phase shifts, such as turf algae outcompeting kelp forests (e.g. Connell & Russell, 2010; Connell *et al.*, 2013).

Competition and foraging in mobile species is not so straight forward, since they will be competing for food more than space. This is where the complex interplay of processes begins. The feeding rates of consumers will be associated with resource availability and its susceptibility to climate change, competition, predation risk, and the physiologically-induced costs associated with ocean acidification and warming.

For herbivores, resource availability may be enhanced by future climate change (e.g. Russell *et al.*, 2013), and the grazers may be able to elevate their feeding rates, enabling them to overcome any energetic costs associated with ocean acidification and warming. However, altered carbonate chemistry also affects the production of plant phenolics, which are often utilised as herbivore deterrents (as well as UV protection and antimicrobials), and hence influence the quality and palatability of plants (Arnold *et al.*, 2012; Rossoll *et al.*, 2012), as well as plant-herbivore interactions. Changes in plant quality may be exhibited through a greater C:N ratio (van de Waal *et al.*, 2009; but see, Gutow *et al.*, 2014), meaning that herbivores may be required to increase their feeding rates in order to maintain the same energetic intake. Herbivory represents an important ecological process for controlling, for instance, the dominance of macrophytes (Diaz-Pulido *et al.*, 2011), however, any increase in foraging rates from the herbivores will result in greater intensity in the competition with other herbivores (Gaylord *et al.*, 2015).

Predator-prey interactions may also be influenced through changes (for either the predator or prey) in shell properties, body size, tissue mass, immune function, or behaviour (Kroeker *et al.*, 2014). As ocean acidification and warming increase the energetic costs of maintenance, it may result in thinner or weaker shells for some prey (McDonald *et al.*, 2009a; Talmage & Gobler, 2010; Thomsen *et al.*, 2010) making them more susceptible to predation, and reduce handling time or effort for the predator.

Reductions in prey size is a common response to environmental stress (Crim *et al.*, 2011; Gaylord *et al.*, 2011; Waldbusser *et al.*, 2013), and this would result in individuals of smaller size for a given age. Smaller prey could be more susceptible to predators due to reduced handling times (dependent on the predator), and they may require a longer period (or be unable) to achieve a size refuge, whereby they are sufficiently large that their predator is no longer able to handle them (Thompson, 1975). Predators may preferentially choose smaller prey (due to the reduced handling time), but will have to increase their per capita consumption in order to sustain their energetic intake (e.g. Sanford *et al.*,

2014).

It is likely to be energetically costly for all species to maintain acid-base homeostasis in response to ocean acidification (Sokolova *et al.*, 2012), and warming will often lead to increased metabolic rates (Brown *et al.*, 2004). These general increases in maintenance may result in 'trophic heat' (sensu Trussell & Schmitz, 2012) which is to say, the energy loss from a system that would otherwise be available for consumption, and a reduced allocation of energy into growth across all trophic levels (Daufresne *et al.*, 2009). As a result, trophic heat can strongly limit the important ecosystem function of energy transfer to higher trophic levels.

These direct effects of climate change will also occur to the predator and influence predator-prey dynamics. Attenuations in the energy moving up a food chain could result in reduced body sizes for the predator (Trussell *et al.*, 2003, 2006; Miller *et al.*, 2014). With many predator-prey interactions being size-structured (Paine, 1976), changes in the size of the predator may influence their preferred prey size or prey type (Kroeker *et al.*, 2014). Reductions in predator size could limit the maximum prey size that they are able to capture (Holling, 1959).

Different predatory feeding mechanisms may also influence the sensitivity of predators to future climate change (Kroeker *et al.*, 2014). For instance, predators that require muscle or claw strength (e.g. crabs, starfish) may be required to reallocate additional energy to maintain this ability. For example, ocean acidification has been reported to reduce crab claw strength (Landes & Zimmer, 2012) and cause muscle wastage in a brittle star (Wood *et al.*, 2008). In contrast, foraging by predatory gastropods that utilise drilling (through alternating phases of active rasping and acidic dissolution; Chétatl & Fournié, 1969), is not associated with their own shell defences (Amaral *et al.*, 2012), and their chitinous radulas are not likely to be directly affected by ocean acidification or warming (but see Marchant *et al.*, 2010, which reported radula damage following short-term exposure to ocean acidification).

Generalist predators should be capable of expanding their dietary breadth, or even changing their preferred prey type, should the energetic value or abundance of the prey be reduced (Gaylord *et al.*, 2015). This may, however, be more of an issue for specialist predators, since their responses should be more closely linked with basic energetic constraints from the consumption of their preferred prey (e.g. Sanford *et al.*, 2014).

### Non-consumptive effects

Predators will not only influence their prey population through ingestion, they can also impose predation risk and cause them to modify their traits, such as behaviour, life-history decisions and morphology (Preisser *et al.*, 2005). These trait-mediated interactions (Abrams, 1995; Werner & Peacor, 2003) result from a trade-off between foraging success and predation risk (Lima & Dill, 1990; Lima & Bednekoff, 1999). Hence, predation risk will often determine where and when prey choose to forage. For example, a prey may detect a predator and remain in their refuge rather than foraging, or only forage on those resources that are protected, such as in crevices or shaded rock overhangs (Matassa & Trussell, 2011). This will result in resources being consumed at a quicker rate near refuge (weak non-consumptive effects), leaving resources further away intact

(strong non-consumptive effects). Therefore, non-consumptive effects may strongly dictate the spatial and temporal distribution of resources (Matassa & Trussell, 2011).

In addition to predation risk influencing the energetic intake of prey (through influences on foraging (e.g. Trussell *et al.*, 2003, 2006), it can also cause stress-induced energetic trade-offs. For instance, prey may produce a thicker shell in response to predator chemical cues (e.g. Trussell, 1996; Trussell & Nicklin, 2002), or in the case of bivalve species, increase their byssal thread production, allowing them to increase their attachment strength to the substrate (e.g. Leonard *et al.*, 1999). In some situations, ocean acidification and warming can diminish these responses, likely intensifying the vulnerability of prey to predation. In the gastropod *Littorina littorea* (L., 1758), highly elevated  $p\text{CO}_2$  conditions (13842 ppm) attenuated the shell thickening response to predation cues (Bibby *et al.*, 2007). Similarly, ocean acidification weakens the proteinaceous byssal threads that anchor mytilid mussels to the substrate (O'Donnell *et al.*, 2013), possibly requiring additional production of the energetically costly byssal threads (Babarro *et al.*, 2008).

Changing environments can also modulate behaviour by interfering with sensory inputs and neural functioning. For example, elevated levels of  $p\text{CO}_2$  is hypothesised to re-model the sensory pathway of the GABA-A system of marine organisms, including the larval clownfish (*Amphiprion percula* Lacepède, 1802), damselfish (*Neopomacentrus azysron* Bleeker, 1877), and gastropod *Gibberulus gibbosus* (Röding, 1798) (Nilsson *et al.*, 2012; Watson *et al.*, 2014), causing sensory and behavioural impairment, including learning ability (Chivers *et al.*, 2014). This phenomenon is thought to be associated with ion regulatory mechanisms during high  $p\text{CO}_2$  exposure (accumulation of intracellular  $\text{HCO}_3^-$  and  $\text{Cl}^-$ ) which interfere with neurotransmitter functions (for more details, see Nilsson *et al.*, 2012). Impaired learning regarding the identity of predators during high  $p\text{CO}_2$  (Chivers *et al.*, 2014), or diminished detection of the olfactory cues for settlement (Munday *et al.*, 2009a), or reduced chemosensory detection of food (e.g. Free Ocean Carbon Dioxide Enrichment (FOCE) experiment, Barry *et al.*, 2014), for instance, will negatively affect the survivorship of the individual.

Mesocosm-based studies have indicated that ocean acidification can modify top-down impacts on resources (e.g. grazing invertebrates on biofilms, Russell *et al.*, 2013), and yet the indirect effects of climate change may be at least as strong as direct effects. In a paper by Alsterberg *et al.* [2013], it was demonstrated that the direct (positive) effects of ocean acidification and warming on the ecosystem functioning of primary producers were mediated by the presence of grazing invertebrates. Similar positive effects of elevated  $\text{CO}_2$  (at the  $\text{CO}_2$  seeps of Ischia, Italy) on primary producers were shown to benefit the highly productive seagrass, *Posidonia oceanica* (Linnaeus 1758), and through changes in canopy structure and food availability, provide refuge to its associated communities from future ocean acidification (Garrard *et al.*, 2014). Predicting the effects of ocean acidification and warming on communities is challenging because although an overall loss of biodiversity is expected (e.g. Fabricius *et al.*, 2011, 2014; Kroeker *et al.*, 2011, 2012), both direct and (less predictable) indirect effects (e.g. reduced habitat complexity) are likely to result in complex outcomes, but must be considered to provide realistic predictions for marine communities.

## 1.5 EFFECTS OF ANTHROPOGENIC CO<sub>2</sub> ON BIODIVERSITY-ECOSYSTEM FUNCTIONING

Anthropogenic influences are altering the composition of biological communities and increasing rates of species extinctions and the invasion of non-native species (Byrnes *et al.*, 2014), which can subsequently alter ecosystem properties and the goods and services they provide to humanity (Hooper *et al.*, 2005). Research has demonstrated that (in general) more species-rich assemblages are more productive and efficient in their resource use when compared to assemblages that contain fewer species (Byrnes *et al.*, 2014). There is no expectation that ecosystems are going to abruptly transform in response to ocean acidification and warming, rather that they will go through a series of consecutive changes due to the species-specific responses of organisms to elevated CO<sub>2</sub> and temperature (Gattuso & Hansson, 2011).

Currently, the exact mechanisms of how future climate change will influence biodiversity-ecosystem functioning in the marine environment are not clear. However, it is likely to primarily occur through alterations to the principal mediators of complementarity and/or dominance. Complementarity can be considered as local deterministic processes which increase the performance of a community beyond what is expected from the performance of individual species raised alone (e.g. facilitation or niche differentiation, Hooper *et al.*, 2005; Finke & Snyder, 2008). For example, Griffin *et al.* [2008] found predator diversity effects attributed to inter-specific competition (within a polyculture of predatory crabs) that more than doubled their resource capture (due to resource partitioning) compared to the monoculture treatments. Dominance effects relate to local and regional stochastic processes involved in community assembly (Loreau *et al.*, 2001), and are associated with the recruitment of a few key, highly productive (and hence, locally dominant) species. The dominance diversity effect arises from the proposal that with an increasing number of species, it is more likely that assemblage will contain those highly productive key species (Aarssen, 1997; Huston, 1997; Tilman *et al.*, 1997).

Ocean acidification and warming may influence complementarity through a number of ways. Assuming optimum foraging theory, the physiological responses of the consumer may result in increased per-capita feeding rates to meet the energetic costs of living in warmer, acidified waters. This could increase diversity effects by limiting resources (with diversity effects exhibited through niche partitioning), with any negative, direct effects on the resource (e.g. reduced body size, Daufresne *et al.*, 2009) possibly enhancing diversity effects further. Should resources benefit from ocean acidification or warming (e.g. elevated C<sub>T</sub> increasing productivity in autotrophs), then consumer diversity effects could be reduced (or even absent) as resources become more available. Moreover, some basal resources, such as biofilm or macroalgae, will undergo biodiversity-ecosystem functioning relationships themselves, and form a feedback loop to influence consumer complementarity.

Future conditions predicted with climate change may influence dominance diversity effects because of the diverse species-specific effects (Harvey *et al.*, 2013; Kroeker *et al.*, 2013b). Any positive or negative effects on the key, highly productive species may promote or diminish dominance effects, and moreover, those species are not necessarily going to remain dominant in future conditions. The different physiological tolerances of individuals means changes to intra- and interspecific interactions (see section 1.4.2), and

the 'winners' and 'losers' of climate change may determine the hierarchy of dominance, and subsequently dictate ecosystem functioning.

It is likely that a larger number of species will be required to buffer ecosystem processes against the predicted temporal variability of ecosystems in response to a changing environment. Climate change will likely lead to a cascade of ecological effects due to the responses at the individual (section 1.3), population (section 1.4.1), community (section 1.4.2) and ecosystem (this section). In order to achieve a more holistic picture of the response at the community and ecosystem levels will require an understanding of how factors at different levels of biological hierarchy will influence responses to climate change. Only then can we establish whether the future organisational structure of marine ecosystems will resemble the communities of today, and how the goods and services of future ecosystems will be affected.

## 1.6 STRUCTURE OF THIS THESIS

Studies of the biological effects of ocean acidification and warming on marine organisms in isolation have provided some insight into the sensitivity of species to these changing conditions (Gattuso *et al.*, 2009). However, since these stressors are unlikely to operate independently (Halpern *et al.*, 2008b), there is now a need to understand how the combined effects of temperature and acidification will affect marine biota. In Chapter 2, a meta-analytical synthesis of the literature will be used to explore the interactions between ocean acidification and warming, how these drivers will affect marine biological responses, and whether variation in biological responses can be explained by different life-history characteristics.

Experimental evidence has suggested that due to the species-specific responses of ocean acidification and warming, the ecological interactions between organisms are also likely to be affected (Kordas *et al.*, 2011). However, these drivers are not often tested in combination and therefore in Chapter 3, ocean carbonate chemistry and warming (replicating end of the century conditions) will be manipulated in aquarium-based mesocosms in order to test the effects of ocean acidification and increased temperature on predator-prey interactions.

Understanding in the role of the predator on rocky intertidal community structure has often focussed on the effect that predator consumption rates has on prey-density ('lethal effects', see Chapter 3). However, predators are also capable of influencing prey traits (eg. behaviour or morphology; Trussell *et al.*, 2003) indirectly, in a process termed non-consumptive effects or trait-mediated indirect interactions (TMIs; Abrams, 1995). Therefore in Chapter 4, aquarium-based mesocosms will be used again to investigate the ability of predators to influence prey-traits which, along with the direct physiological responses of the prey, will be used to investigate how community dynamics on rocky intertidal shores will be influenced in warmer, acidified future oceans.

As ocean acidification and warming influences interspecific relationships, these changes, in turn, can drive important local scale changes in community dynamics, biodiversity and ecosystem functioning. Environmental changes, such as climate change, means that ecosystems are being subjected to a rapid and widespread loss of species, it is crucial to understand the consequences that such a biodiversity loss will have in the functioning

of marine ecosystems. In [Chapter 5](#), an aquarium-based experiment will be used to investigate whether the diversity of three epilithic invertebrate grazers will alter ecosystem functioning differently under future climate change scenarios.

In [Chapter 6](#), *in situ* experiments will be carried out at the volcanic CO<sub>2</sub> seeps of Vulcano, Italy. This experiment will use pre-established stations along the natural gradient, which, depending on their proximity to the vents, are either at normal pH levels (8.1-8.2) or at lowered pH levels (7.8-7.9). This chapter will use a comparison between these stations to investigate the effects that ocean acidification will have on the physiology, ecology and population dynamics of a predatory gastropod (*Hexaplex trunculus*).

Finally, in [Chapter 7](#), the results of the thesis will be brought together, along with a discussion into the insights gained as a result of the findings presented in this thesis.

## META-ANALYSIS REVEALS COMPLEX MARINE BIOLOGICAL RESPONSES TO THE INTERACTIVE EFFECTS OF OCEAN ACIDIFICATION AND WARMING

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### **Abstract**

Ocean acidification and warming are considered two of the greatest threats to marine biodiversity, and yet the combined effect of these stressors on marine organisms remains largely unclear. A meta-analytical approach was used to assess the biological responses of marine organisms to the effects of ocean acidification and warming in isolation and combination. As expected biological responses varied across taxonomic groups, life-history stages and trophic levels, but importantly, combining stressors generally exhibited a stronger biological (either positive or negative) effect. Using a subset of orthogonal studies we show that four out of five of the biological responses measured (calcification, photosynthesis, reproduction and survival, but not growth) interacted synergistically when warming and acidification were combined. The observed synergisms between interacting stressors suggest that care must be made in making inferences from single-stressor studies. Our findings clearly have implications for the development of adaptive management strategies particularly given that the frequency of stressors interacting in marine systems will be likely to intensify in the future. There is now an urgent need to move towards more robust, holistic and ecologically realistic climate-change experiments that incorporate interactions. Without them accurate predictions about the likely deleterious impacts to marine biodiversity and ecosystem functioning over the next century will not be possible.





## 2.1 INTRODUCTION

The concentration of atmospheric carbon dioxide (CO<sub>2</sub>) has increased from 280ppm in pre-industrial times to a present day level of 391ppm (Le Quéré *et al.*, 2012). Over the last 100 years this has led to changes in global sea surface temperatures (+0.74 °C) and ocean carbonate chemistry (Orr *et al.*, 2005), which have included ocean acidification by 0.1 pH units (Caldeira & Wickett, 2003; Kleypas *et al.*, 2006). By the year 2100 sea-surface temperatures are expected to rise by a further 1-4 °C while increased CO<sub>2</sub> (aq) will result in the decreased availability of carbonate ions and a further reduction in pH by 0.3-0.5 units (Caldeira & Wickett, 2005; IPCC, 2007a; Gooding *et al.*, 2009). These changes in temperature and ocean carbonate chemistry are considered two of the greatest threats to marine biodiversity (Kleypas *et al.*, 1999; Doney *et al.*, 2009), leading to changes in the physiological performance of individual organisms, which will in turn alter biotic interactions, community structure and ecosystem functioning.

A range of marine biological responses have already been observed in response to ocean warming including coral bleaching (Hoegh-Guldberg *et al.*, 2007), species range shifts (Parmesan & Yohe, 2003; Root *et al.*, 2003), changes to phenology (Walther *et al.*, 2002), and reduced organism body size (Daufresne-2009). Experimental manipulations simulating predicted future ocean temperatures have suggested that warming will also lead to increased metabolic costs for plants and animals (O'Connor *et al.*, 2009), increased consumption rates (Sanford 1999) and changed food-web structures (Petchey *et al.*, 1999). Observed responses of marine organisms to recent ocean acidification are limited (but see Iglesias-Rodriguez *et al.*, 2008b; Moy *et al.*, 2009), but are expected to become increasingly apparent in the next 50-100 years (Doney *et al.*, 2009; Feely *et al.*, 2009). Experimental evidence, however, suggests that responses are likely to be varied (Hendriks *et al.*, 2010; Kroeker *et al.*, 2010) and will include hypercapnic suppression of metabolism (Christensen *et al.*, 2011), acid-base balance disturbances (Miles *et al.*, 2007), plus both positive and negative effects on skeleton formation (related to a decrease in carbonate saturation; Doney *et al.*, 2009; Ries *et al.*, 2009).

The vulnerability of marine species and ecosystems to temperature, in particular, is well established (for reviews; Hoegh-Guldberg & Bruno, 2010; Richardson *et al.*, 2012; Wernberg *et al.*, 2012). Conversely, the resilience of marine organisms to ocean acidification still remains a reasonably challenged concept (Dupont *et al.*, 2010a; Hendriks *et al.*, 2010). Recent meta-analyses assessing the biological effects of ocean acidification (Dupont *et al.*, 2010a; Hendriks *et al.*, 2010; Kroeker *et al.*, 2010) concurred that it is unlikely to act in a uniform manner as variation exists in marine organism responses and resilience. Hence if any meaningful comparisons are to be made on the response of marine organisms, they need to be hypothesis-driven based on *a priori* assigned groupings, such as taxonomic groups or life stages (Dupont *et al.*, 2010a).

Studies of the biological effects of elevated temperature and acidification on marine organisms in isolation have provided some insight into the potential tolerance of species to these changing conditions (Gattuso *et al.*, 2009). However, given that these stressors are unlikely to operate independently (Halpern *et al.*, 2007), there is now a need to attain a more ecologically realistic understanding of how the combined effects of temperature and acidification will affect marine biota (Sala *et al.*, 2000; Fabry *et al.*, 2008). This is vital in order to inform future adaptive management strategies. Other recent meta-

analyses, across ecological systems, have also shown that multiple stressors can lead to non-additive interactions with responses dependent on the type of stressor as well as the level of ecological organisation investigated (e.g. population vs. community, autotroph vs. heterotroph) (Crain *et al.*, 2008; Darling & Côté, 2008; Tylianakis *et al.*, 2008). Moreover, the mechanism through which the stressor acts upon the organism will affect the response. Multiple stressors acting through a similar pathway may have an additive effect (Crain *et al.*, 2008). In contrast, any stress-induced tolerances could lead to antagonisms (Blanck, 2002), while those stressors that act on different, but dependent mechanisms may act synergistically (Kneitel & Chase, 2004). These reviews did however contain few, if any, studies that investigated both warming and acidification. Therefore, the concurrent effect of temperature and ocean acidification via elevated CO<sub>2</sub> remains unclear, but is likely to lead to complex biological outcomes.

Organisms vary widely in their individual responses to ocean warming and acidification as a result of differences in their physiological and ecological characteristics (Dupont *et al.*, 2008; Fabry, 2008). For example, many marine organisms possessing a calcium carbonate (CaCO<sub>3</sub>) structure would be considered more susceptible to ocean acidification as this process will impair their capacity to produce calcified skeletons (Doney *et al.*, 2009). Conversely, some species, including some calcified species, will have the capacity to buffer against the deleterious effects of acidification by utilising acid-base compensation (Claiborne & Evans, 1992; Larsen *et al.*, 1997), active mobility and metabolism (Widdicombe & Spicer, 2008; Whiteley, 2011) or energy reallocation (Wood *et al.*, 2008; McDonald *et al.*, 2009a). Warmer temperature, up to a limit, stimulates metabolism in ectotherms, resulting in faster growth and development (Byrne *et al.*, 2011). Moreover, it has been speculated that warming could even ameliorate the negative impacts of acidification (McNeil *et al.*, 2004; Kleypas & Yates, 2009).

Species responses to ocean warming and acidification are also likely to vary among life-history stages (Byrne, 2011). Early life-history stages are considered most susceptible to changes in both temperature and ocean acidification (Byrne, 2011). These stressors may, however, have positive and/or negative effects for the successful recruitment of juveniles to the adult population. Trophic level is also likely to determine how species respond due to differences in environmental sensitivity (Petchey *et al.*, 2004; Raffaelli, 2004). Previous work has suggested the effects of multiple stressors are likely to act antagonistically in autotrophs, but synergistically in heterotrophs (Crain *et al.*, 2008). Furthermore, since higher trophic levels contain less 'biological insurance' (*sensu* Yachi & Loreau, 1999), i.e. less taxonomic, physiological, and genetic diversity, they are predicted to be more susceptible to multiple environmental perturbations (Christensen *et al.*, 2006) which could act upon them synergistically (Crain *et al.*, 2008).

Using a meta-analytical approach of the peer-reviewed literature this chapter assessed the impacts and interactions of ocean acidification and warming on marine biological responses. Given that variability in the strength and direction of responses was expected, data were classified according to taxonomic groups, calcifiers and non-calcifiers, life-history stage and level of trophic organisation (autotroph and heterotroph) in terms of changes in rates of calcification, growth, photosynthesis, reproduction and survival. Specifically, this chapter aimed to address three questions: (i) How do marine organisms respond to warming and acidification in isolation? (ii) How do marine organisms respond to

the combined effects of warming and acidification? (iii) How do warming and acidification impacts interact?

## 2.2 MATERIAL AND METHODS

### 2.2.1 *Data selection and suitability criteria*

Searches for peer-reviewed articles in which studies explicitly investigated climate change using either elevated temperature, ocean acidification or elevated temperature and acidification were carried out using ISI Web of Science [v 5.8] © and Google Scholar using the following keywords: ocean warming, global warming, ocean acidification, hypercapnia, climate change and combinations therein. In addition this chapter used the European Project on Ocean Acidification (EPOCA) blog (<http://oceanacidification.wordpress.com/>), citation searches; analysis of reference lists in comprehensive reviews (Hendriks *et al.*, 2010; Kroeker *et al.*, 2010; Wernberg *et al.*, 2012), and then cross-referenced with the bibliographies of identified articles.

The analysis was limited to studies published between 1<sup>st</sup> January 1990 and 1<sup>st</sup> January 2012, as the majority of experimental climate change studies that manipulated climate change conditions in line with IPCC AR1 predictions and subsequent updates (IPCC, 1990, 2007a) were published post 1990. Only controlled manipulative experiments were used for analysis. In addition, the control treatments of the environmental stressor (eg. pH, CO<sub>2</sub>, or temperature) needed to represent current ambient levels and were based on the authors' opinion of 'ambient'. The experimental organisms had to be subjected to elevated temperature alone, acidification alone, or both warming and acidification. When studies included environmental variables in addition to temperature and ocean acidification (such as light availability or nutrients), these responses were only considered at 'ambient' levels as determined by the authors'. To explore predicted future conditions for 2100, the manipulation treatments needed to conform to the IPCC IS92a "business-as-usual" emission scenario for the year 2100 (IPCC, 2007a). Studies that manipulated carbonate chemistry using acid addition were omitted, because it does not reproduce the changes in HCO<sub>3</sub><sup>-</sup> concentration that occur as a result of increased CO<sub>2</sub> (aq) (Iglesias-Rodriguez *et al.*, 2008a,b). Finally, only studies that reported a measureable biological response were included.

As response variables, calcification (or dissolution) rates, growth, photosynthesis, reproduction and survival (mortality was converted to survival by using 1 - mortality) were used. There were insufficient data on other response variables (e.g. feeding rates, metabolism) to allow quantitative analysis. A number of articles included more than one species, response, location, or treatment level and all were included in the analysis if they met the suitability criteria. This ensured that a broad range of responses could be fully explored, despite lessening the independence of the data from that particular study (Gurevitch *et al.*, 1992). To maintain independence of data we included only one response, chosen at random, from studies reporting several responses that could be classified in the same category (e.g. growth expressed as changes in length and biomass). Derived metrics from studies that included time-series data were based on the final time point of exposure. To investigate inherent biological variability, records were categorised according to taxonomy, life-history stage, level of trophic organisation (autotroph, heterotroph) and whether the organism possessed a CaCO<sub>3</sub> skeletal structure.

To enable a calculation of effect size, studies that met our initial criteria could only be used if they reported a mean response value, some form of variance (standard deviation, standard error or confidence interval), and a sample size. In some instances data were extracted from graphical images in publications and in these situations data were extracted using the program GraphClick (v. 3.0) (Neuchatel, Switzerland).

### 2.2.2 Data analysis

Biological responses to ocean warming and acidification were measured for each experiment to establish the proportional change between the control and treatment means using response ratios. In their original metric response ratios were weighted towards positive responses, so the response ratios were log transformed to maintain symmetry in the analysis and ease the biological interpretation (Hedges *et al.*, 1999). We chose a log response ratio (lnRR), over other methods, to estimate the effect size because of the high capacity to detect true effects and their robustness to small sample sizes (Lajeunesse & Forbes, 2003).

Analyses were carried out using the R (version 2.15.1; R Development Core Team, 2012) package 'Metafor' (Viechtbauer, 2010). We selected a weighted random-effects model to estimate a summary effect size. Random-effects analysis assumes that the true effect size differs between experiments and the estimated summary effect is the mean of the effects observed across the studies. This meant that even if studies had a low weighting, the individual effect sizes from all of the studies could be incorporated into the summary effect (Borenstein *et al.*, 2009). This ensured that the biological variation inherent in the responses was properly accounted for. Both the within-study variance (inverse of the effect size variance) and the between-study variance ( $\sigma^2_{\text{pooled}}$ ) were used to weight the studies. Therefore studies with higher replication and/or lower variance were considered more precise and weighted accordingly (Hedges & Olkin, 1985). Between-study variance was estimated using the DerSimonian Laird method (DerSimonian & Laird, 1986).

Statistical significance was attributed to each summary effect size by calculating a bias-corrected 95% confidence interval (CI) (see Hedges & Olkin, 1985) and comparing it with zero. If the summary effect size did not overlap zero then it was considered to be significantly different. A total heterogeneity statistic (Q) was used to ascertain that the variation observed was a combination of both true variation (between studies) and random error (within studies) (Borenstein *et al.*, 2009). This was tested as the observed weighted sum of squares against a chi square distribution with  $n - 1$  degrees of freedom, using the null hypothesis that observations shared a common effect size.

Combinations of the treatment effect (CO<sub>2</sub>/pH, temperature, temperature and CO<sub>2</sub>/pH) and response variables (calcification, growth, photosynthesis, reproduction, and survival) were used as the comparison groups in all analyses. Separate exploratory analyses were also used to test the differences between *a priori* defined groups using a mixed-effects model (see Viechtbauer, 2010). It was appreciated that this form of multiple exploratory analyses on the same dataset could be prone to Type I error. Hence we used these analyses to identify the underlying patterns of the biological responses. The categorical moderators used were the different taxonomic groups (corals, crustaceans, crustose coralline algae, echinoderms, fishes, non-calcifying algae, molluscs, phytoplankton and

seagrasses), calcifying and non-calcifying organisms, developmental stages (embryos, larvae, juveniles and adults), and trophic organisation (autotroph and heterotroph). This process applied a summary effect size and 95% CI to each of the different categories for comparison. To formally test for differences between these categories, a test for heterogeneity ( $Q_M$ ) was used; this identified total heterogeneity explained by that particular categorical moderator (Gurevitch *et al.*, 1992). A significant  $Q_M$  indicated that there was a difference between the categories.

The taxonomic group of phytoplankton was initially divided into coccolithophores, cyanobacteria, diatoms, dinoflagellates and foraminifera, however, results were pooled after detecting no difference using a test for heterogeneity ( $Q_M$ ). When assigning a 'trophic organisation' to each observation, we defined autotrophs as any organism capable of producing organic carbon-based compounds from inorganic sources, through either photosynthesis or chemosynthesis. For example, corals were designated as autotrophic as photosynthesis is generally regarded as their principal mode of carbon acquisition (Hoogenboom *et al.*, 2006; Mass *et al.*, 2007). Over all of the meta-analytical results, the summary effect sizes were not reported if there were fewer than five studies available for analysis, and categorical moderators were not reported if there were fewer than three studies. This was a pragmatic decision to ensure that a broad range of responses could be assessed, as some categories only had a few studies that met our criteria. Therefore, the categorical analyses did not always include all the observations from the full model.

### 2.2.3 Interactions between multiple stressors

Interaction strength between ocean warming and acidification was ascertained according to the methods for factorial meta-analysis (Gurevitch *et al.*, 2000; Crain *et al.*, 2008). To be included, studies needed to be controlled factorial experiments reporting four outcomes of acidification [ $Y_{acid}$ ], warming [ $Y_{temp}$ ], acidification and warming [ $Y_{both}$ ], and a control treatment [ $Y_{ct}$ ] (Underwood, 1997). Therefore, not all of the observations from the full model could be analysed. Multiple observations from the same study were included if separate factorial results were provided. The interaction strength ( $\ln RR_{int}$ ) and individual effects ( $\ln RR_{acid}$  and  $\ln RR_{temp}$ ) of each study were then calculated as:

$$\ln RR_{int} = \ln \left( \frac{Y_{both}}{Y_{temp}} \right) - \ln \left( \frac{Y_{acid}}{Y_{ct}} \right) \quad (2.1)$$

$$\ln RR_{acid} = \left( \frac{Y_{acid}}{Y_{ct}} \right) \quad (2.2)$$

$$\ln RR_{temp} = \left( \frac{Y_{temp}}{Y_{ct}} \right) \quad (2.3)$$

, where  $Y$  is the biological response for the indicated group (in subscript). The sampling variance of  $\ln RR_{int}$  is:

$$s_N^2(\ln RR_{int}) = s^2 \frac{1}{N_{ct}(Y_{ct})^2} + \frac{1}{N_{acid}(Y_{acid})^2} + \frac{1}{N_{temp}(Y_{temp})^2} + \frac{1}{N_{both}(Y_{both})^2} \quad (2.4)$$



, where  $N$  is the sample size and  $Y$  is the biological response for the indicated group (in subscript), and  $s$  is the pooled standard deviation.

Although less conservative than an additive model (Folt *et al.*, 1999), we used a multiplicative model to calculate the interactions since the underlying model of the metric  $\ln RR$  is multiplicative (Hawkes & Sullivan, 2001; Morris *et al.*, 2007), and this model is also thought to be more biologically realistic (Sih *et al.*, 1998). Synergisms occur when the cumulative response of a stressor ( $Y_{\text{both}} - Y_{\text{temp}}$ ) is greater than the stressor in isolation ( $Y_{\text{acid}} - Y_{\text{ct}}$ ), and antagonistic when the cumulative impact is smaller than expected (Folt *et al.*, 1999). Therefore, the interaction type was classified as multiplicative (i.e. the null hypothesis) if the interaction effect size and 95% confidence interval overlapped zero. If the direction of both individual stressors was positive, the interaction was considered synergistic when the interaction effect size was greater than zero, and antagonistic if less than zero. Similarly, when the direction of the individual stressors was either both negative or had opposite signs, then the interpretation was reversed (i.e. synergies occurred when the interaction effect size was less than zero).

#### 2.2.4 Sensitivity analyses and publication bias

Sensitivity analysis was used to investigate the influence of any experimental study that demonstrated an unusually large effect size. This was achieved in a step-wise manner by ranking each experiment by the magnitude of effect size, removing the largest one, and re-running the analysis. Likewise, if any study contributed five or more observations to a category, the study was omitted and the analyses re-run. If studies were considered to be driving the results, then they were omitted from the analysis of that response variable.

The number of studies with an effect size of zero that would be required to change the results of the meta analysis from significant to non-significant ('file drawer problem') was determined using Rosenthal's failsafe number (Rosenthal, 1979). It was decided that if five or less studies (of zero effect size) were required to change the effect size, then that categorical analysis was not considered robust.

## 2.3 RESULTS

### 2.3.1 Overall biological responses

Out of 196 peer-reviewed articles that investigated the biological responses of marine organisms to ocean warming and/or acidification 107 met our criteria, giving 623 unique observations (Table A.1). Observations that did not meet the selection criteria are listed in Table A.2, and the results from all the heterogeneity tests for overall within-effects ( $Q$ ) and between categories ( $Q_M$ ) are reported in Table A.3.

Meta-analysis of the whole dataset revealed that calcification and reproduction were negatively affected by ocean acidification and unaffected by ocean warming. In contrast, the independent effects of ocean acidification and warming had no effect on growth or photosynthesis, while both ocean acidification and warming had significant negative effects on survival (Figure 2.1).

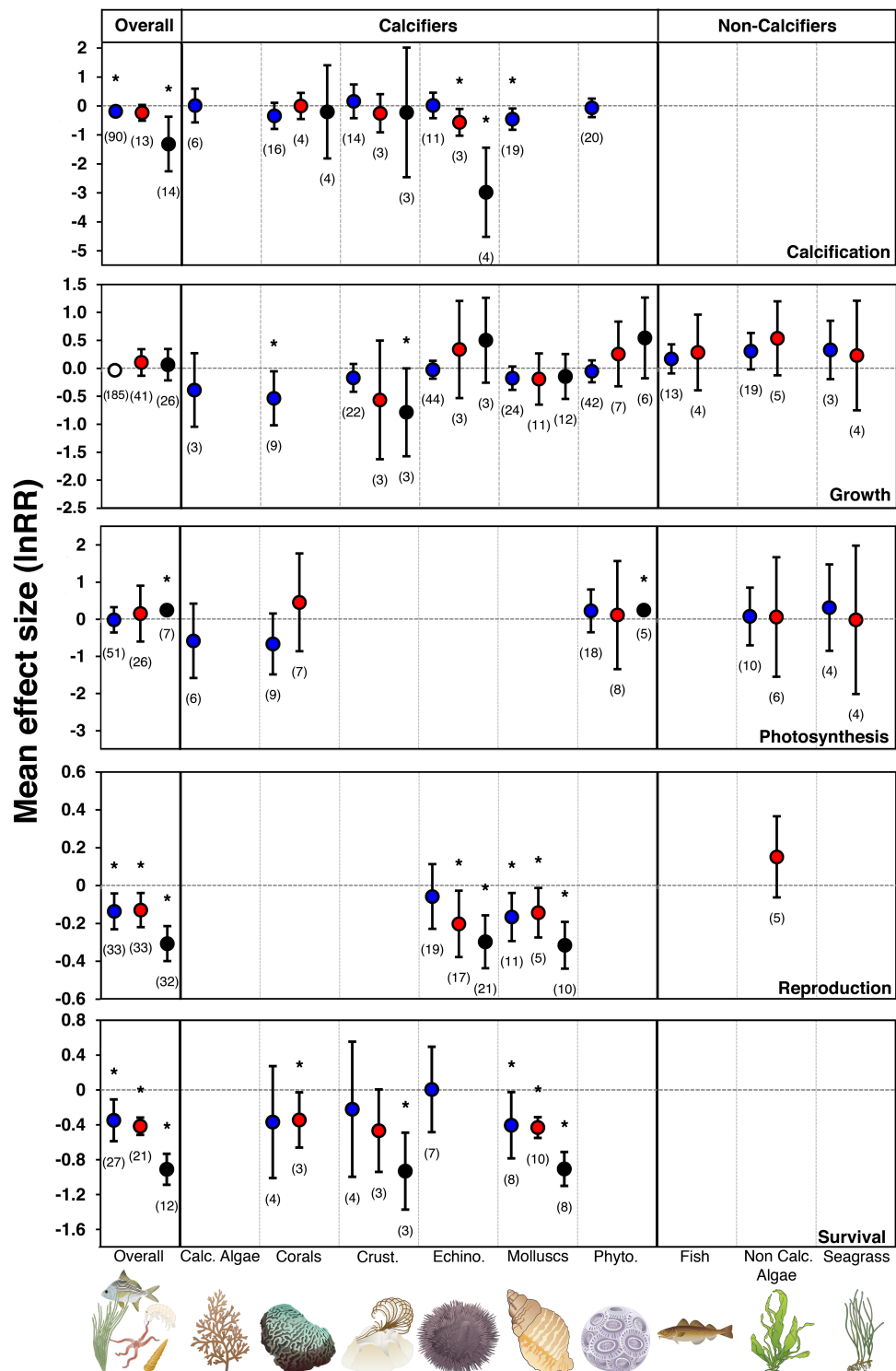


Figure 2.1: The mean effect of ocean acidification (blue circles), ocean warming (red circles), and combined ocean acidification and warming (black circles) on calcification, growth, photosynthesis, reproduction and survival for different taxonomic groups. The mean log response ratio and  $\pm$  95% confidence intervals are shown for overall (combined results), calcifiers (calcifying algae, corals, crustaceans, echinoderms, molluscs and phytoplankton) and non-calcifiers (fishes, non-calcified algae, seagrass). The number of observations in each analysis is shown in parentheses. The zero line indicates no effect, and significance (\*) of mean effects is determined when the  $\pm$  95% confidence interval does not overlap zero

Combined warming and acidification had a significant negative effect on calcification, survival and reproduction with the magnitude of response greater than that observed for the stressors in isolation (Figure 2.1). Concurrent acidification and warming had no effect on growth overall, but had a significant positive effect on photosynthesis in autotrophs (Figure 2.1).

Significant within-group heterogeneity was observed in all of the responses (Table A.3), therefore in order to quantify patterns in the literature regarding the effects of warming and acidification, we made comparisons between the *a priori* groupings (taxonomic groups, calcifiers and non-calcifiers, life-history stage and level of trophic organisation) for each biological response.

### 2.3.2 Calcification

Although the independent effects of ocean acidification and warming on calcification were varied, we did not detect differences between taxonomic groups (Figure 2.1; Table A.3), life-history stages (Figure 2.3; Table A.3) or trophic organisation (Figure 2.4; Table A.3). Conversely, the combined effects of ocean warming and acidification on calcification varied significantly by taxonomic group (Figure 2.1;  $p < 0.001$ ; Table A.3), with echinoderms more negatively affected than either corals or crustaceans (both  $p < 0.001$ ); life-history stage (Figure 2.3;  $p = 0.0397$ ; Table A.3) with juveniles more negatively affected compared to adults ( $p = 0.040$ ); and trophic organisation (Figure 2.4;  $p = 0.010$ ; Table A.3) with heterotrophs being negatively affected by warming and acidification, while autotrophs were neutrally affected (Figure 2.4).

### 2.3.3 Growth

Despite overall growth responses being unaffected by ocean acidification and warming, we detected differences in the combined effects between taxonomic groups (Figure 2.1;  $p < 0.001$ ; Table A.3). Crustaceans and molluscs were both significantly negatively affected, while echinoderms and phytoplankton were positively affected (Figure 2.1). The combined effects of warming and acidification varied with trophic organisation (Figure 2.4;  $p < 0.001$ ; Table A.3), with autotrophs being positively affected and heterotrophs negatively affected (Figure 2.4). Similarly, ocean warming also varied between taxonomic groups (Figure 2.1;  $p < 0.001$ ; Table A.3), with echinoderms, phytoplankton, fishes and macroalgae positively affected, and molluscs negatively affected (Figure 2.1); and trophic organisation (Figure 2.4;  $p = 0.004$ ; Table A.3) with a positive effect on autotrophs and a neutral effect on heterotrophs. Ocean warming also positively affected non-calcifiers (Figure 2.2). The independent effects of ocean acidification varied by taxonomic group (Figure 2.1;  $p < 0.001$ ; Table A.3), with crustose coralline algae, corals, molluscs and phytoplankton negatively affected, crustaceans and echinoderms unaffected, and fishes, macroalgae and seagrass positively affected (fishes and macroalgae  $p < 0.05$ , seagrass  $p = 0.06$ ); and calcifiers/non-calcifiers (Figure 2.2;  $p < 0.001$ ; Table A.3) with the calcifying organisms exhibiting generally negative effects and non-calcifiers generally positive effects (Figs. 1 and 2). No differences in growth responses were detected between life stages with any of the stressors (Figure 2.3).



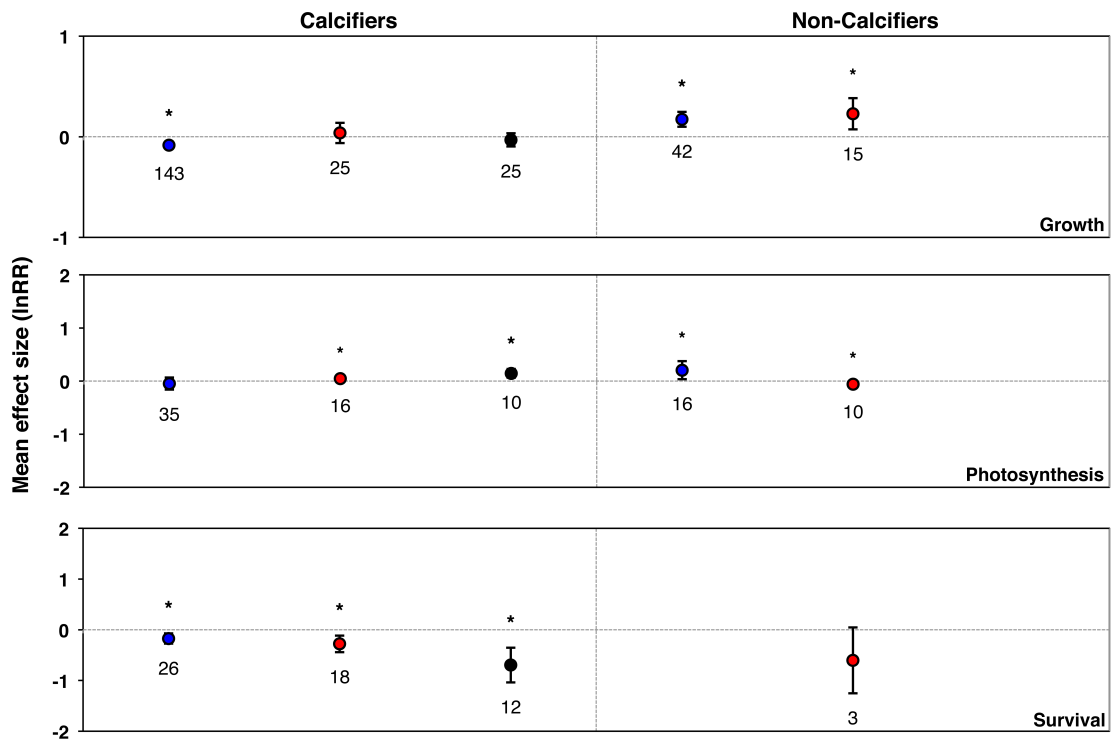


Figure 2.2: The mean effect of ocean acidification (blue circles), ocean warming (red circles), and combined ocean acidification and warming (black circles) on growth, photosynthesis and survival for calcifying and non-calcifying organisms. The mean log response ratio and  $\pm$  95% confidence intervals are shown for calcifiers and non-calcifiers. The number of observations in each analysis is shown in parentheses. The zero line indicates no effect, and significance (\*) of mean effects is determined when the  $\pm$  95% confidence interval does not overlap zero.

#### 2.3.4 Photosynthesis

We did not detect differences in the effects of combined warming and acidification on photosynthesis between either taxonomic groups (corals and phytoplankton; Figure 2.1), calcifiers/non-calcifiers (Figure 2.2), or life stages (Figure 2.3), despite finding significant differences between these *a priori* groupings in both acidification and warming independently (Table A.3). We did, however, find that in combination significant positive effects were observed in phytoplankton (Figure 2.1) and calcifying organisms (Figure 2.2).

Similarly, ocean warming positively affected photosynthesis in phytoplankton and calcifying organisms, but had a negative effect on both macroalgae (Figure 2.1) and non-calcifying organisms (Figure 2.2). The independent effects of ocean acidification on photosynthesis also varied by taxonomic group (Figure 2.1;  $p < 0.001$ ; Table A.3), with corals being negatively affected, and both phytoplankton and seagrass positively affected. Ocean acidification also positively affected photosynthesis in non-calcifying organisms (Figure 2.2).

#### 2.3.5 Reproduction

The effects of warming and acidification, both combined and independently, on reproduction were varied between the taxonomic groups (Figure 2.1; all  $p < 0.001$ ; Table A.3).

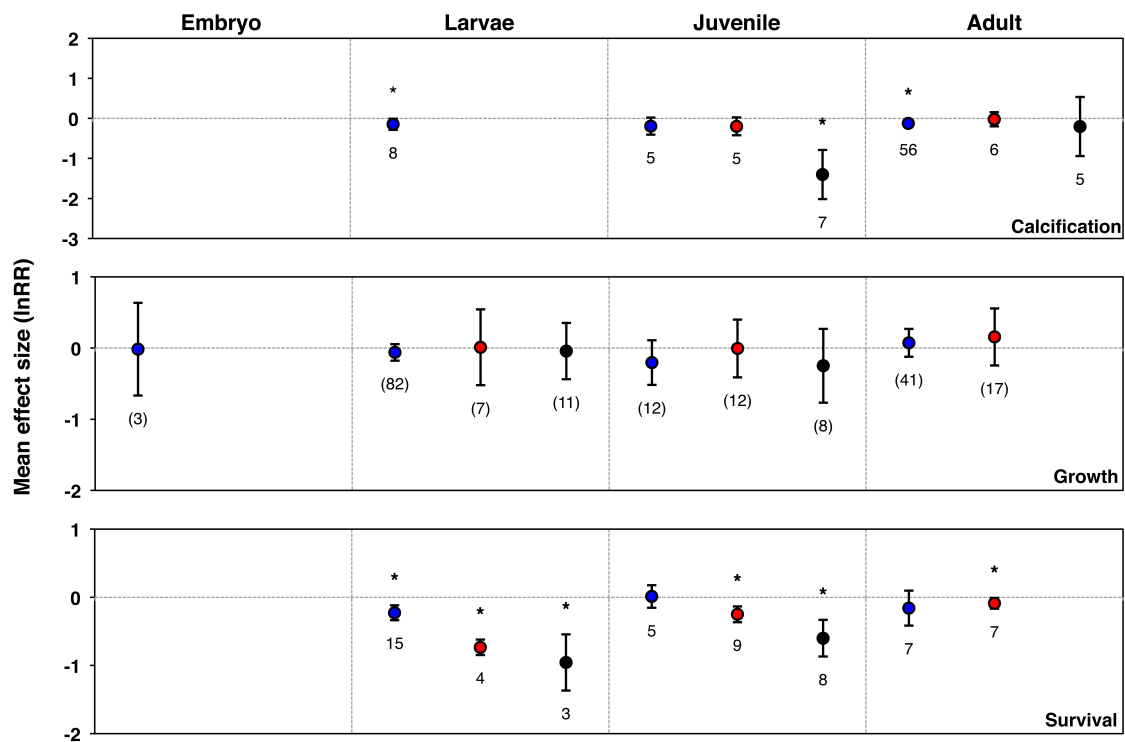


Figure 2.3: The mean effect of ocean acidification (blue circles), ocean warming (red circles), and combined ocean acidification and warming (black circles) on calcification, growth and survival in different life-stages. The mean log response ratio and  $\pm$  95% confidence intervals are shown for embryos, larvae, juveniles and adults. The number of observations in each analysis is shown in parentheses. The zero line indicates no effect, and significance (\*) of mean effects is determined when the  $\pm$  95% confidence interval does not overlap zero.

In response to ocean warming alone positive responses by crustaceans and macroalgae and autotrophs were observed while heterotrophs responded negatively ( $p = 0.0071$ , Table A.3). In all three combinations of stressor, echinoderms were unaffected whilst molluscs were negatively affected.

### 2.3.6 Survival

The combined effects of warming and acidification resulted in significant negative responses across the categories, but only varied by taxonomic group (Figure 2.1;  $p = 0.030$ ; Table A.3). Specifically, combined acidification and warming had a larger negative effect on molluscs than crustaceans (Figure 2.1;  $p = 0.029$ ). Independently, the effects of ocean warming on survival also varied by life stage (Figure 2.3;  $p < 0.001$ ; Table A.3) with a larger negative effect on juveniles compared to adults ( $p = 0.023$ ) and on larvae compared to both juveniles and adults (both  $p < 0.001$ ). Additionally, ocean warming also had a negative effect on molluscs (Figure 2.1), calcifiers (Figure 2.2), and both autotrophs and heterotrophs (Figure 2.4). Survival responses to ocean acidification in isolation did not vary between taxonomic group, calcifiers/non-calcifiers, life-stage or trophic organisation. However, ocean acidification did negatively affect corals and molluscs (Figure 2.1), calcifiers (Figure 2.2), larvae (Figure 2.3) and autotrophs (Figure 2.4).

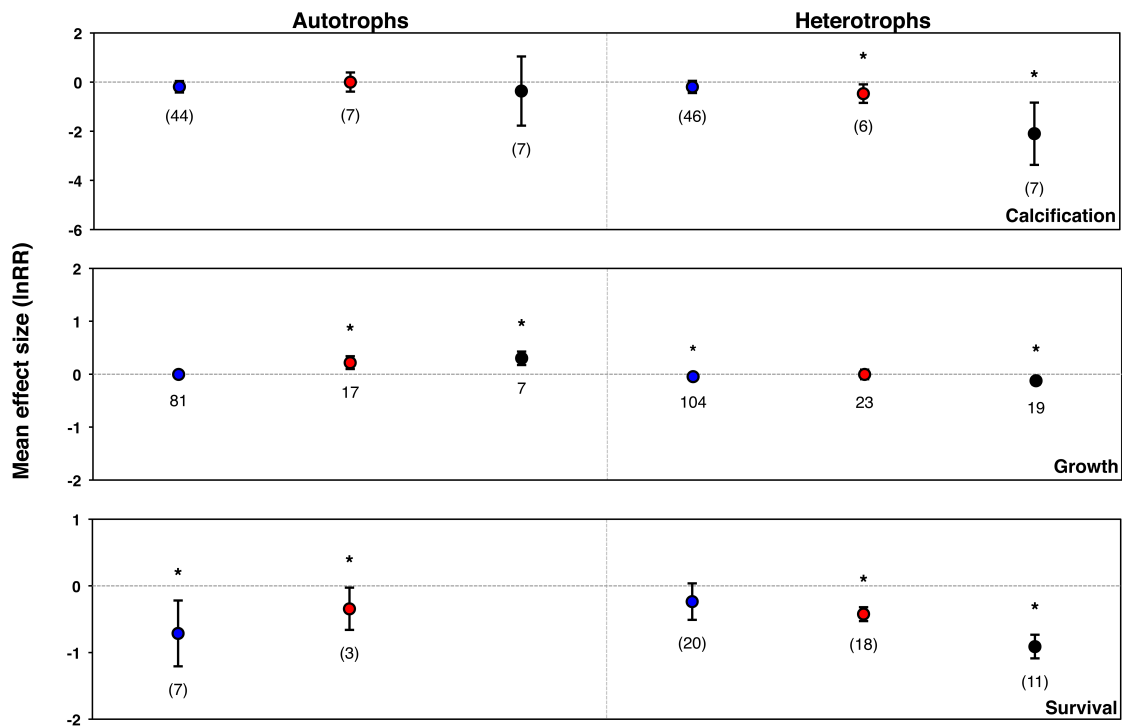


Figure 2.4: The mean effect of ocean acidification (blue circles), ocean warming (red circles), and combined ocean acidification and warming (black circles) on calcification, growth, photosynthesis, reproduction and survival for different levels of trophic organisation. The mean log response ratio and  $\pm$  95% confidence intervals are shown for autotrophs and heterotrophs. The number of observations in each analysis is shown in parentheses. The zero line indicates no effect, and significance (\*) of mean effects is determined when the  $\pm$  95% confidence interval does not overlap zero.

### 2.3.7 Interactions between multiple stressors

Interaction strength between ocean warming and acidification was determined for the subset of fully-factorial studies, and therefore did not include all of the observations from the full model. Significant synergistic interactions were observed for calcification ( $z = 3.69$ ,  $p < 0.001$ ), photosynthesis ( $z = 2.23$ ,  $p = 0.026$ ), reproduction ( $z = 2.97$ ,  $p = 0.003$ ), and survival ( $z = 3.04$ ,  $p = 0.002$ ), but not for growth ( $z = 1.56$ ,  $p = 0.117$ ).

### 2.3.8 Sensitivity analyses and publication bias

To test the robustness of our analyses against large effect sizes, we removed each comparison step-wise and re-ran each analysis, omitting experiments if they changed the significance of either heterogeneity or the mean effect size of the response variables. This resulted in twelve experiments being omitted from subsequent analyses across several treatment-response variable scenarios (see Table A.2 for more detail). We used Rosenthal's fail-safe number to assess the importance of potential publication bias and found that our response variables were robust, with the lowest value being 358 additional studies required to change the effect size (based on an original experiment quantity of 11). No individual study contributing more than five experiments changed the significance of either the heterogeneity or mean effect size of the response variables.

## 2.4 DISCUSSION

To test the robustness of our analyses against large effect sizes, we removed each comparison step-wise and re-ran each analysis, omitting experiments if they changed the significance of either heterogeneity or the mean effect size of the response variables. This resulted in twelve experiments being omitted from subsequent analyses across several treatment-response variable scenarios (see Table A.2 for more detail). We used Rosenthal's fail-safe number to assess the importance of potential publication bias and found that our response variables were robust, with the lowest value being 358 additional studies required to change the effect size (based on an original experiment quantity of 11). No individual study contributing more than five experiments changed the significance of either the heterogeneity or mean effect size of the response variables.

### 2.4.1 *Effects of ocean acidification*

Ocean acidification generally had an adverse effect on a large range of marine biota with more specific differences between life-history characteristics. Calcifying organisms were generally negatively affected by ocean acidification, with significant biological variability in responses, such as molluscs being negatively affected, which may be due to their poor ion-regulation and inability to buffer their internal compartments (Fabry *et al.*, 2008; Widdicombe & Spicer, 2008; Melzner *et al.*, 2009; Dupont *et al.*, 2010a). Conversely, crustaceans were generally unaffected by acidification perhaps due to their active mobility, higher metabolism and capacity to control intracellular pH (Gaillard & Malan, 1983; Widdicombe & Spicer, 2008; Whiteley, 2011).

Overall, less is known about how non-calcifying organisms are likely to respond to acidification (Connell & Russell, 2010), particularly marine fishes (Ishimatsu, 2005; Munday *et al.*, 2009c), however, our results show that non-calcifiers were generally unaffected by acidification and for growth were positively affected when analysed together as a group. Fishes are thought to have more efficient acid-base regulation compared to invertebrates (Widdicombe & Spicer, 2008), which, when coupled with an increased food intake or reduced energy expenditure (Munday *et al.*, 2009c), could explain the positive growth response observed. Similarly, non-calcifying marine autotrophs demonstrated an increased growth to ocean acidification. This is possibly via their capacity to derive dissolved inorganic carbon from the increased CO<sub>2</sub> (aq) (Beer & Koch, 1996).

Autotrophs may be capable of increasing their inorganic carbon assimilation (Rost & Riebesell, 2004) and buffering the negative effects on calcification (Ries *et al.*, 2009). However, negative effects on growth were observed in calcifying autotrophs. Our results suggest that the negative effects on calcifying autotrophs may outweigh positive effects associated with the increased availability of CO<sub>2</sub> (aq) as a substrate for photosynthesis. We also show some evidence that calcification and survival in early life-history stages was more negatively affected by ocean acidification, highlighting not only the susceptibility of early life-stages, but also the subtle nature of life-history responses when compared to species-specific effects of heterogeneity (Kurihara, 2008; Kroeker *et al.*, 2010). Importantly our results agree with the findings from previous meta-analyses (Dupont *et al.*, 2010a; Kroeker *et al.*, 2010) while introducing a further 48 key studies (168 additional data points).

### 2.4.2 *Effects of ocean warming*

Moderate elevations in temperatures will increase metabolic rates (Hochachka & Somero, 2002), which influences key biological processes that regulate life-history characteristics (O'Connor *et al.*, 2009). While marine organisms are capable of acclimation to a range of temperatures, once their thermotolerance limits are exceeded, organism fitness is reduced and the risk of mortality increases (Hofmann & Todgham, 2010; Tomanek, 2010). We found that both non-calcifying organisms and autotrophs demonstrated increased growth under warming conditions, likely due to this increase in metabolic rate (Hochachka & Somero, 2002). Warming, however, had no effect on the growth of heterotrophs and even negatively affected their calcification. Possibly because the metabolism complexes of autotrophs (photosynthesis-limited) are less sensitive to ocean warming than the respiration-limited metabolism of heterotrophs (Lopez-Urrutia *et al.*, 2006). Moreover, our results also support the hypothesis that the threshold for deleterious warming may vary between developmental stages (Byrne *et al.*, 2009, 2010) with survival significantly lower in larvae than in juveniles, and significantly lower in juveniles than in adults.

### 2.4.3 *Simultaneous acidification and warming*

Meta-analysis of the full dataset revealed that the combined stressors caused significant negative effects on calcification, reproduction and survival, and a significant positive effect on photosynthesis, but no effect on growth. Importantly, we also found that four out of the five responses (calcification, photosynthesis, reproduction and survival) showed a synergistic interaction between acidification and warming. Although, such synergistic interactions between stressors (i.e. where the outcome was greater than the sum of the individual stressors, Folt *et al.*, 1999) are relatively common (e.g. Sala *et al.*, 2000; Harley *et al.*, 2006), they are concerning because they are also unpredictable. Hence, such synergies limit our capacity to predict potential future impacts from single-stressors studies.

Ecological synergies are important to marine systems (Paine *et al.*, 1998; Harley *et al.*, 2006; Sutherland *et al.*, 2006) because they can further exacerbate adverse effects and reduce ecosystem resilience (Folke *et al.*, 2004). They can also introduce indirect effects via biotic interactions (Darling & Côté, 2008; Tylianakis *et al.*, 2008). For example, climate-driven changes in plankton communities can regulate top predators through bottom-up control (Beaugrand *et al.*, 2003). Moreover, since marine systems are subject to multiple interacting stressors (Halpern *et al.*, 2007), it is possible that the addition of further stressors would introduce additional adverse consequences (e.g. Przesławski *et al.*, 2005). Therefore, our results highlight the need to move away from single-stressor studies and towards more ecologically realistic research incorporating multiple stressors, in order to more fully understand how near-future anthropogenic change will affect marine biodiversity.

There was, as expected, variation in our analyses amongst the biological responses to combined warming and acidification between different taxonomic groups, calcifiers and non-calcifiers, trophic levels, and life-history stages. The combination of warming and acidification generally exhibited a stronger effect (either positive or negative) than when exposed to the stressors in isolation. For instance, echinoderms are highly vulnerable to ocean acidification (Dupont *et al.*, 2010c), likely due to their skeletons being formed from highly soluble magnesium calcite (Politi *et al.*, 2004). However, the addition of moderate

increases in temperature (+4 °C), as predicted for the end of the century, resulted in further adverse effects (e.g. [Byrne \*et al.\*, 2011](#)), such as the highly negative calcification responses shown here. In some instances, the combined effects of warming and acidification were reduced compared to the individual effects. Corals had both calcification and photosynthesis negatively affected by ocean acidification in isolation, while they were unaffected by the combined effects, suggesting that the addition of warming may ameliorate the adverse effects of acidification ([McNeil \*et al.\*, 2004](#); [Kleypas & Yates, 2009](#)). These differences in the resilience of marine organisms will have important implications for ecosystem level responses.

Interestingly, the combined effect of warming and acidification positively affected growth in echinoderms, which could be explained by energy allocation, where the cost of homeostatic regulation can be influenced by changes in somatic and reproductive growth performance ([Melzner \*et al.\*, 2009](#)). Hence, if more energy is utilised to maintain growth, then calcification responses could be more adversely affected (e.g. [Arnold \*et al.\*, 2009](#); [McDonald \*et al.\*, 2009a](#)). Other studies have shown an alternative strategy for energy allocation where growth was negatively affected, while calcification was maintained (e.g. [Wood \*et al.\*, 2008](#); or crustaceans, this study), highlighting the species-specific nature of biological responses.

Heterotroph responses to ocean acidification and warming individually differed, with acidification reducing growth, but not affecting calcification or survival; while warming alternatively did not affect growth, reducing both calcification and survival responses. However, the energetic demands of the combined effects of acidification and warming on heterotrophs resulted in calcification, growth and survival all being reduced. Conversely, the combined effects of warming and acidification positively affected growth in autotrophs, likely due to the effect of temperature on metabolic rate ([Hochachka & Somero, 2002](#)), while CO<sub>2</sub>, acted as a substrate for photosynthesis and possibly indirectly promoted growth (e.g. phytoplankton; [Loehle, 1995](#)). Moreover, since multiple stressors affecting autotrophs are likely to act antagonistically ([Crain \*et al.\*, 2008](#)), and the dissolved inorganic carbon sources utilised by marine autotrophs is set to increase ([Raven \*et al.\*, 2005](#)), photosynthesising organisms will likely be more resilient to conditions predicted for the end of the century; as long as they do not exceed their thermotolerance ([Hofmann & Todgham, 2010](#); [Tomanek, 2010](#)) and are not limited by other factors, such as inorganic nutrient availability ([Langdon & Atkinson, 2005](#); [Cohen & Holcomb, 2009](#); [Ries \*et al.\*, 2009](#)).

Our results also show that the combined effects of warming and acidification had significant negative effects on the survival of early life-history stages. Although there was insufficient data to compare across all life-history stages, both larvae and juveniles were highly susceptible to changes in temperature and ocean acidification, supported by previous research ([Gosselin & Qian, 1997](#); [Hunt & Scheibling, 1997](#)). Indirectly, ocean warming can reduce the mortality of larvae by shortening the planktonic duration ([Lamare & Barker, 1999](#)), when they are most vulnerable to predation ([O'Connor \*et al.\*, 2007](#)). Our results, however, identified that the combined effects of ocean acidification and warming increased mortality, indicating that multiple stressors will have important implications for population persistence, potentially acting as a bottle-neck for some species ([Dupont \*et al.\*, 2010c](#); [Byrne, 2011](#)).

#### 2.4.4 Conclusions

Quantitative syntheses of the published literature can provide powerful inferences, however, like all analyses they are subject to caveats. We identified and incorporated the available literature that met our selection criteria, however, this also outlines the current gaps in knowledge and highlights opportunities for further work. Moreover, species-specific sources of heterogeneity are always likely to make some results from the literature greatly context-dependent (e.g. [Fabry, 2008](#); [Kurihara, 2008](#); [Dupont \*et al.\*, 2010a](#); [Hendriks \*et al.\*, 2010](#); [Kroeker \*et al.\*, 2010](#)). Hence, our findings highlight the complexity of marine organism responses to ocean warming, acidification and their interaction. The magnitude, direction and interaction of the effects varies between response type, likely a result of the pathways driving the biological response. Responses also differ between taxonomic groups, trophic levels and life-history stages. Most importantly, we observed synergistic interactions between ocean acidification and warming in four of the five biological responses measured (calcification, photosynthesis, reproduction and survival), highlighting the difficulties in making inferences from single stressor studies. However, single-factor studies in junction with those that manipulate multiple stressors can play a vital role in understanding the pathways through which particular stressors operate and will enable a more accurate assessment of the likely outcomes of interactions between warming and acidification. Importantly, we must also consider further abiotic and biotic stressors in the marine environment that are likely to also interact with warming and acidification ([Halpern \*et al.\*, 2007](#)) as well as scaling up studies from individuals and populations to communities and ecosystems ([Harley \*et al.\*, 2006](#)). Such large-scale multi-factorial experiments would not only increase our knowledge of the functioning and resilience of marine ecosystems, but provide explicit evidence to policymakers on the effectiveness of conservation and management strategies in response to future environmental change.





## OCEAN WARMING AND ACIDIFICATION PREVENTS COMPENSATORY RESPONSE IN A PREDATOR TO REDUCED PREY QUALITY

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### Abstract

Ocean acidification and warming have the potential to negatively impact many organisms by influencing their ability to maintain positive life-history traits. While there is increasing evidence for the impacts of climate change at the individual-level, much less is known about how species' likely idiosyncratic responses may alter ecological interactions. Using an aquarium-based mesocosm experiment, we demonstrate that ocean acidification and warming not only directly alter species (individual) physiological performance, but also their predator-prey dynamics. Our results demonstrate that tissue production in the acorn barnacle *Semibalanus balanoides* was reduced under realistic scenarios of future climate change, and hence their energetic quality as the principal prey resource for the dogwhelk *Nucella lapillus* was diminished. However, rather than increasing their feeding rates as a compensatory mechanism, consumption rates of *N. lapillus* were reduced to the point that they exhibited starvation (a loss of somatic tissue), despite prey resources remaining abundant. The resilience of any marine organism in response to stressors is fundamentally linked to their ability to obtain and assimilate energy. Therefore, our findings suggest that the cost of living under future climate change may surpass the energy intake from consumption rates, which is likely exacerbated through the bottom-up effects of reduced prey quality. If, as our results suggest, changes in trophic transfer of energy are more common in a warmer, high CO<sub>2</sub> world, such alterations to the predator-prey dynamic may have negative consequences for the acquisition of energy in the predator and result in energetic trade-offs. Given the importance of predator-prey interactions in structuring marine communities, future climate change is likely to have major consequences for community composition and the structure and function of natural ecosystems.



### 3.1 INTRODUCTION

Ecosystems are structured by physical processes and interactions between co-existing species (McCann *et al.*, 1998; Halpern *et al.*, 2008b; Berlow *et al.*, 2009). The strength and direction of these biotic interactions can determine the stability of populations and communities (De Ruiter *et al.*, 1995; Rooney *et al.*, 2006; Neutel *et al.*, 2007; O’Gorman & Emmerson, 2009). Idiosyncratic responses to projected ocean acidification and warming (Harvey *et al.*, 2013; Kroeker *et al.*, 2013b), due to differences in species physiological tolerances and performance (Pörtner, 2012), are likely to alter the outcomes of competitive (e.g. Diaz-Pulido *et al.*, 2011; Connell *et al.*, 2013) and trophic interactions (e.g. O’Connor, 2009; Ferrari *et al.*, 2011; Alsterberg *et al.*, 2013; Poore *et al.*, 2013). Hence, accurately predicting the consequences of climate change first requires understanding of how local-scale changes to the environment can drive changes in community dynamics, and the flow of energy in ecosystems.

Our understanding of the impacts of ocean acidification and warming on individual species is increasing rapidly (see Byrne, 2011; Wicks & Roberts, 2012; Gazeau *et al.*, 2013; Harvey *et al.*, 2013; Koch *et al.*, 2013; Brodie *et al.*, 2014 for reviews), but empirical data on how these abiotic stressors affect species interactions remains limited (but see Connell & Russell, 2010; Alsterberg *et al.*, 2013; Poore *et al.*, 2013; Russell *et al.*, 2013; Sanford *et al.*, 2014). The resilience of any heterotrophic marine organism to the combined effects of ocean acidification and warming is fundamentally linked to their ability to obtain and assimilate energy. Any changes in the consumption rates of a consumer due to abiotic stressors will not only directly alter the abundance of their prey (or resource), it will affect how much energy is transferred up the food chain. The effects of climate stressors on basal resources can therefore indirectly affect higher order consumers through bottom-up effects (e.g. Beaugrand *et al.*, 2003), or even allow organisms to maintain homeostatic physiological compensation if their food supply is sufficient (e.g. Melzner *et al.*, 2011). Understanding the simultaneous responses of species at different trophic levels to environmental stressors will therefore better enhance our ability to predict patterns at more complex levels of organisation, such as entire communities and ecosystems.

Organisms are expected to incur increased energetic expenditure associated with climate change as a function of altered metabolic demands (Pörtner, 2010), acid-base physiology (Miles *et al.*, 2007; Fabry *et al.*, 2008) and calcification mechanisms (e.g. Gattuso & Buddemeier, 2000; Ries *et al.*, 2009). In order to balance energy intake and expenditure, consumption rates would be expected to increase (e.g. Sanford *et al.*, 2014). The efficiency of energy transfer between trophic levels reflects the remaining energy following maintenance and growth (Jennings & Mackinson, 2003), that can be passed along the food chain. Thus, any greater energetic demands due to climate change, including warming and acidification, may result in constraints on the efficiency of trophic energy transfer (Dossena *et al.*, 2012). Moreover, any direct disruptions to feeding rates (Siikavuopio *et al.*, 2007; Russell *et al.*, 2013) or changes in digestion efficiency (Stumpp *et al.*, 2013) in response to climate change, will likely cause complex changes in the energy allocation of individuals (e.g. McDonald *et al.*, 2009b; Stumpp *et al.*, 2012b), and could also have important implications for the trophic transfer of energy.

Here, we experimentally tested the effects of elevated  $p\text{CO}_2$  and temperature on the energetic states of the rocky-shore intertidal predator, the dogwhelk (*Nucella lapillus* L. 1758),

and its principal prey, the acorn barnacle (*Semibalanus balanoides* L. 1767), in order to provide information on the effects of predicted climate change on population dynamics and predator-prey interactions, and inform potential impacts for community structure.

## 3.2 MATERIAL AND METHODS

### 3.2.1 Experimental conditions and system design

An orthogonal experimental design was employed with three levels of  $p\text{CO}_2$  (400 ppm, 750 ppm and 1000 ppm) and two levels of temperature (14 °C and 18 °C). Levels of temperature and  $p\text{CO}_2$  were chosen based on the IPCC (IPCC, 2007b) A2 and A1 SRES scenario for the year 2100. These scenarios are approximately equivalent to RCP 6 and RCP 8.5 scenarios, respectively (Rogelj *et al.*, 2012). The experimental system was in a constant temperature room with aquarium heaters (Eheim Jäger 300W) used in the footer tank to maintain elevated temperature treatments. The pH of the mesocosms was set by continuous bubbling of a known air- $\text{CO}_2$  mix (see Findlay *et al.*, 2008).  $\text{pH}_{\text{NBS}}$  (Mettler-Toledo SevenGo Pro pH meter with Inlab 413SG probe, Mettler-Toledo, Germany), temperature and salinity (WTW Cond 3210, WTW, Germany) were recorded every two to three days, and total alkalinity ( $A_T$ , acid-base titration) was measured weekly. In order to calculate the additional carbonate chemistry parameters, following Nisumaa *et al.* [2010], the dissolved inorganic carbon ( $C_T$ ) was calculated using the software CO2SYS (Lewis & Wallace, 1998), with the measured  $\text{pH}_{\text{NBS}}$  and  $A_T$  as the input variables. Subsequently, the additional carbonate system parameters were calculated using the R package seacarb (Lavigne & Gattuso, 2010) using the calculated  $C_T$  and measured  $A_T$  (Table 3.1). For both CO2SYS and seacarb, we used dissociation constants from Mehrbach *et al.* [1973], as adjusted by Dickson & Millero [1987], and  $\text{KSO}_4$  using Dickson [1990] (Table 3.1). Light conditions were adjusted weekly to maintain natural light:dark cycles, seawater was replenished weekly, and fresh water added (every two to three days) to account for any salinity increases due to evaporation.

### 3.2.2 Animal collection and experimental design

*N. lapillus* and *S. balanoides* (on rock chippings) were collected from Borth, Wales, UK (52° 47. 960' N, 4° 05.520' W) during September 2012, and held for three days in a flowing aquarium system. Adult *S. balanoides* and sub-adult individuals of *N. lapillus* (with an active growing shell lip, as classed by Hughes & Taylor [1997]) were chosen. For *N. lapillus*, this is because at this stage, linear shell extension is generally nearing an asymptote, while the thickening of the shell wall, associated with sexual maturation, has not yet begun (Hughes & Taylor, 1997). These species were chosen to represent model, ubiquitous, temperate rocky-shore intertidal species.

Two experiments were carried out. The first experiment maintained *S. balanoides* and *N. lapillus* (separately) under experimental conditions ( $n = 5$ ) in tanks (10 L - 36.5 × 26.5 × 14.5 cm and 4.5 L - 26 × 17 × 18 cm, respectively) for 80 days. Throughout this experiment, both species were fed ad libitum, which for *S. balanoides*, consisted of supplementing unfiltered seawater with a mix of microalgae and zooplankton (including *Artemia* sp. Maréchal & Hellio, 2011) every two to three days. *N. lapillus* were fed *S. balanoides* (from a holding tank under the same experimental treatment).

Table 3.1: Seawater properties during the exposure phase (day 1-80).  $pH_T$ , temperature, salinity, and total alkalinity ( $A_T$ ) are measured values. Seawater  $pCO_2$ , dissolved inorganic carbon ( $C_T$ ), bicarbonate ( $HCO_3^-$ ), carbonate ( $CO_3^{2-}$ ), carbon dioxide ( $CO_2$ ), saturation states for calcite ( $\Omega_{calc}$ ) and aragonite ( $\Omega_{arag}$ ) are values calculated using the carbonate chemistry system analysis program CO2SYS and the R package seacarb. Numbers in parentheses represent S.E.

	$pH_T$	Temp	Salinity	$A_T$	$pCO_2$	$C_T$	$HCO_3^-$	$CO_3^{2-}$	$CO_2$	$\Omega_{calc}$	$\Omega_{arag}$
Target:		(°C)	(psu)	( $\mu\text{mol kg}^{-1}$ )	( $\mu\text{atm}$ )	( $\mu\text{mol kg}^{-1}$ )	( $\mu\text{mol kg}^{-1}$ )	( $\mu\text{mol kg}^{-1}$ )	( $\mu\text{mol kg}^{-1}$ )		
400 Amb.	7.992 (0.004)	14.19 (0.01)	34.93 (1.01)	2067.35 (6.96)	416.33 (2.9)	1894.84 (5.93)	1757.15 (5.16)	121.67 (0.89)	16.02 (0.06)	1.87 (0.01)	2.92 (0.02)
750 Amb.	7.762 (0.003)	14.21 (0.02)	34.04 (0.59)	2118.29 (6.09)	772.21 (5.66)	2027.71 (5.62)	1919.7 (5.23)	78.31 (0.49)	29.69 (0.12)	1.2 (0.01)	1.88 (0.01)
1000 Amb.	7.64 (0.003)	14.01 (0.03)	34.65 (0.68)	2112.56 (5.96)	1042.78 (5.32)	2060.77 (5.58)	1960.52 (5.26)	59.91 (0.35)	40.35 (0.11)	0.92 (0.01)	1.44 (0.01)
400 Warm	8.021 (0.003)	17.96 (0.01)	34.87 (0.44)	2210.77 (6.23)	413.87 (2.96)	1991.32 (5.24)	1821.4 (4.49)	155.68 (0.94)	14.24 (0.06)	2.42 (0.01)	3.74 (0.02)
750 Warm	7.812 (0.004)	17.9 (0.03)	34.47 (0.39)	2336.07 (7.65)	761.06 (5.68)	2202.05 (6.73)	2067 (6.05)	108.83 (0.82)	26.22 (0.11)	1.69 (0.01)	2.62 (0.02)
1000 Warm	7.686 (0.004)	18.12 (0.02)	34.44 (0.48)	2278.89 (8.05)	1020.62 (6.84)	2191.5 (7.42)	2074.08 (6.89)	82.47 (0.6)	34.95 (0.13)	1.28 (0.01)	1.98 (0.01)

The second experiment was a feeding trial, using individuals of *S. balanoides* and *N. lapillus* taken from the first experiment (described fully in section 3.2.6). In brief, rock chips of *S. balanoides* (~300 individuals) were placed into tanks (2 L - 22.5 × 16.5 × 7.8 cm) for 28 days under (the same) experimental conditions, as paired-replicates ( $n = 5$ ) with *N. lapillus* either present (one individual) or absent (control). The food supply for *S. balanoides* during the second experiment was maintained as above.

### 3.2.3 Prey growth, calcification and survival

Following the completion of experiment one *S. balanoides* growth rates and survival were ascertained by comparing the digital images (Nikon PowerShot A2300 HD) taken at the start and end of the first experiment (similar to Findlay *et al.*, 2010). Length-mass relationships were established for *S. balanoides* to relate the barnacle rostro-carinal diameter (RCD, mm) with both dry body tissue mass (mg) and shell mass (mg) (see Goll  ty *et al.*, 2008). We found a significant relationship between the barnacle size (RCD length) and both their body mass ( $R^2 = 0.803$ ,  $p < 0.0001$ ), and shell mass ( $R^2 = 0.688$ ,  $p < 0.0001$ ). Therefore changes in RCD (final RCD - initial RCD) over the experiment were used to describe changes in tissue production and calcification. Starting tissue and shell mass were initially established by length-mass relationship (dry body tissue mass, mg =  $0.1026 \times \text{RCD}^{3.0137}$  mm,  $n = 100$ , and shell mass, mg =  $1.3982 \times \text{RCD}^{2.7390}$  mm,  $n = 100$ ) using barnacles collected at the same time as the experimental organisms. Since we expected treatment-specific changes in the length-mass relationships, final measurements used treatment-specific length-mass relationships (Figures B.2-B.7 for tissue mass and Figures B.9-B.14 for shell mass), which were established by sacrificing a sub-sample of barnacles (ash free dry mass 6 hrs, 500 °C) that were collected across replicates from each treatment at the completion of the two experiments. Survival was measured as the remaining percentage of barnacle individuals (compared to the starting abundance) after the first experiment. Following Findlay *et al.* [2010], prior to photography, the ability of barnacle individuals to close their operculum was tested, with any individual unable to close their operculum classed as dead.

### 3.2.4 Predator growth and calcification

Before the first experiment, each experimental *N. lapillus* ( $n = 12$  per tank) was labelled with a numbered, coloured queen bee tag (EH Thorne Ltd, Rand, UK) and measured for starting shell length (digital calliper,  $\pm 0.1$ mm), and dry shell mass and wet body mass using the non-destructive methodology of Palmer (1982). These measurements were repeated at the end of the experiment, and (individual) shell length was measured as final length - initial length, while dry shell growth (i.e. net calcification) and wet body growth were calculated as final mass - initial mass. Dry shell mass was calculated using linear regression with buoyant mass (previously calculated by destructively sampling individuals collected at the same time and place as the experimental individuals; dry shell mass =  $1.6317 \times \text{buoyant mass} - 0.0949$  g,  $n = 50$ ,  $R^2 = 0.9895$ ,  $p < 0.001$ ). Wet body mass was calculated by subtracting the estimated dry shell mass from the total mass of the individual in air. Tissue production was estimated by converting the (initial and final) wet body mass to dry body mass with linear regression (dry body mass =  $0.2142 \times \text{wet body mass} - 0.006$  g,  $n = 50$ ,  $R^2 = 0.9870$ ,  $p < 0.001$ ; this regression was established with the same individuals that were used for the dry shell mass to buoyant mass relationship).

### 3.2.5 Predator standard metabolic rates

Rates of oxygen uptake for *N. lapillus* were measured, as a proxy for resting metabolic rate, using closed bottle respirometers. Individuals were placed in 250 ml respirometers ( $n = 25$ , with five additional blanks, to control for microbial respiration), located in a flowing water bath to maintain their respective temperature and  $p\text{CO}_2$  treatments. The respirometers were covered, but left open for 1 hour to remove any potential handling stress. Before the respirometers were sealed, the starting  $\text{O}_2$  levels were measured using a dissolved oxygen meter (Orion Star A223 DO with polarographic  $\text{O}_2$  electrode, Thermo Scientific, Waltham, MA USA), with the electrode standardised using aerated seawater at 100% saturation. The respirometers were sealed for 2 hours, with the experimental duration chosen in order to maintain  $\text{O}_2$  saturation above 80% and avoid hypoxic conditions (Gnaiger *et al.*, 1989). A second oxygen concentration was measured, and the rates of oxygen uptake were calculated as the difference between the starting and final oxygen concentrations (accounting for microbial respiration). Measurements were then multiplied by the solubility coefficient for oxygen (corrected for temperature and salinity, Harvey, 1955) and the respirometer volume, for standard temperature and (dry) pressure (STPD). Ash-free dry body mass was measured by sacrificing all individuals measured for their metabolic rate (6 hrs at 500 °C), and final oxygen uptake was expressed in terms of  $\mu\text{mol O}_2 \text{ g}^{-1}$  (AFDW)  $\text{h}^{-1}$  STPD.

### 3.2.6 Predator feeding and ingestion efficiency

The amount of *S. balanoides* consumed by *N. lapillus* was quantified by estimating the mass of somatic tissue eaten during the second experiment. The number of *S. balanoides* consumed was measured by changes in the final abundance (with predator present) compared to the starting abundance (Digital photograph; Nikon PowerShot A2300 HD), after subtracting any deceased barnacles in the paired predator-free treatments (i.e. accounting for natural mortality). The somatic tissue was then estimated using the regression between the barnacle RCD and somatic tissue previously established for barnacle tissue production (Figures B.2-B.7). Feeding rates were then converted to energy consumption by converting the estimated somatic tissue of ingested *S. balanoides* to energy units using  $23 \text{ J mg}^{-1}$  (Wu & Levings, 1978).

Ingestion efficiency, the ratio of ingestion to metabolism (Yodzis & Innes, 1992; Vasseur & McCann, 2005; Rall *et al.*, 2010), was also calculated for all the *N. lapillus* individuals used in the feeding trials using the methodology of Vasseur & McCann [2005]. The ingestion efficiency represents the ingestion gain of a consumer relative to metabolic demands, whereby ingestion efficiencies of one indicate that ingestion balances metabolic costs, while ratios below one indicate the metabolic costs exceed ingestion. We used a previously established absorption efficiency (energy assimilated/energy ingested) of 0.4 for *N. lapillus* (Stickle & Bayne, 1987), and converted oxygen uptake into joules by multiplying with an oxycaloric coefficient of  $21.10 \text{ mJ l O}_2^{-1}$ , representing an accepted value for catabolism of carbohydrate (Elliott & Davison, 1975). This value was chosen because most gastropods rely on glycogen stores for energy during activity (Carefoot, 1987).



### 3.2.7 Statistical analysis

Two-way ANOVA was employed to test for possible differences among all measurements (prey growth, calcification and survival; predator growth, calcification, standard metabolic rate, feeding rate and ingestion efficiency) between treatments, with Tukey HSD *post-hoc* tests used to test pair-wise differences. Since tissue production and shell mass were related to RCD (in *S. balanoides*), an ANCOVA was employed to test for differences between treatments in the response of final barnacle tissue and shell mass, using length (RCD) as a covariate, with both length and mass log-transformed. All data was normally distributed (Kolmogorov-Smirnov) as well as displaying homogeneity of variance (Levene) in all cases ( $p > 0.05$ ). Statistical analyses were conducted using R (R Development Core Team, 2012).

## 3.3 RESULTS

### 3.3.1 Prey growth, calcification and survival

Ocean acidification significantly reduced RCD growth in *S. balanoides* ( $F_{2,24} = 12.55$ ,  $p < 0.001$ ; Figure 3.1a), however, growth was not significantly reduced by elevated temperature ( $F_{1,24} = 2.17$ ,  $p = 0.154$ ; Figure 3.1b). After controlling for barnacle length, we observed a significant (negative) effect of both ocean acidification (ANCOVA,  $F_{2,574} = 10.46$ ,  $p < 0.01$ ) and warming (ANCOVA,  $F_{1,574} = 43.46$ ,  $p < 0.001$ ) on barnacle tissue production (i.e. length-mass relationship; see Figure B.1 in the Appendix for regressions), such that *S. balanoides* individuals of the same length had significantly smaller body tissue mass with increasing  $p\text{CO}_2$  concentrations and warming. Hence, both ocean acidification ( $F_{2,24} = 3.85$ ,  $p < 0.05$ ; Figure 3.1c) and warming ( $F_{1,24} = 12.15$ ,  $p < 0.001$ ; Figure 3.1d) significantly reduced barnacle tissue production compared to control conditions. Even after standardising by length, shell mass was not significantly affected by either ocean acidification or warming (see Figure B.8 and Table B.5 in the appendix). As such, barnacle shell mass was not significantly affected by warming ( $F_{1,24} = 0.598$ ,  $p = 0.57$ ), while the (admittedly just non-significant) effect of ocean acidification ( $F_{2,24} = 3.37$ ,  $p = 0.069$ ) was likely due to the decreased (RCD) growth rates exhibited under elevated  $p\text{CO}_2$ , rather than an actual reduction in calcification rates *per se*. Survival rates of *S. balanoides* were significantly lower with both ocean acidification ( $F_{2,24} = 17.54$ ,  $p < 0.001$ ; Figure 3.1e) and warming ( $F_{1,24} = 51.54$ ,  $p < 0.001$ ; Figure 3.1f) when compared to control conditions.

### 3.3.2 Predator growth, calcification and standard metabolic rates

We observed a significant interaction between ocean warming and acidification for tissue production in *N. lapillus* ( $F_{2,24} = 7.47$ ,  $p < 0.001$ ; Figure 3.2). Separately, both elevated temperature ( $F_{1,24} = 37.26$ ,  $p < 0.0001$ ) and the high (1000 ppm)  $p\text{CO}_2$  concentration ( $F_{2,24} = 36.24$ ,  $p < 0.0001$ ) significantly reduced tissue production compared to control conditions (Figure 3.2). When the elevated temperature and high  $p\text{CO}_2$  concentration were combined *N. lapillus* actually experienced negative growth, i.e. a loss of somatic tissue (Figure 3.2). While mortality was not a specific response variable in this experiment there was a higher mortality in these treatments with six *N. lapillus* dying in the high  $p\text{CO}_2$



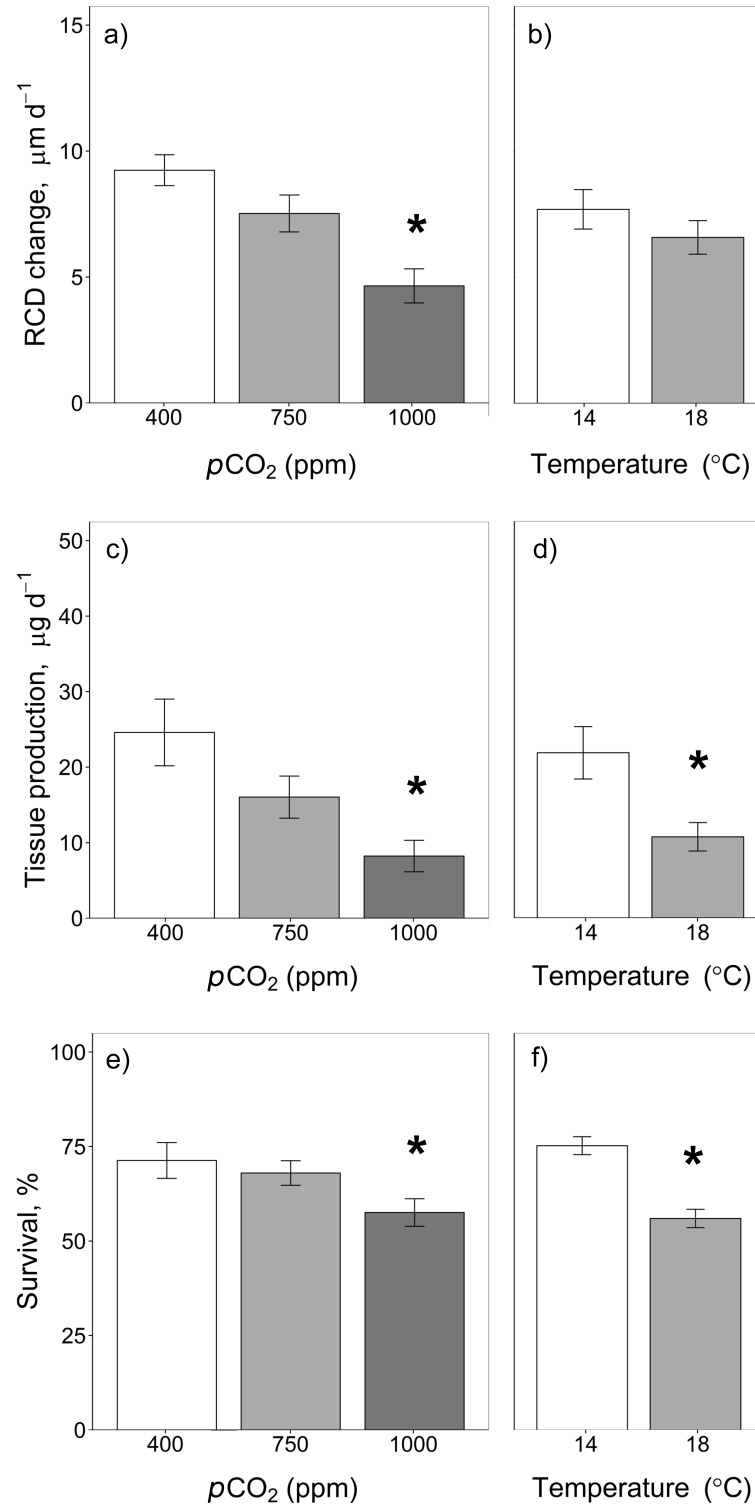


Figure 3.1: (a-b) Mean ( $\pm$  S.E.) rostro-carinal diameter (RCD) change ( $\mu\text{m d}^{-1}$ ), (c-d) Mean ( $\pm$  S.E.) tissue production ( $\mu\text{g d}^{-1}$ ), and (e-f) Mean ( $\pm$  S.E.) survival rate (%) of *S. balanoides*. Panels a, c and e are in response to different  $p\text{CO}_2$  concentrations (400, 750 and 1000 ppm) averaged across levels of temperature. Panels b, d and f are in response to different temperature treatments (14 and 18  $^{\circ}\text{C}$ ) averaged across levels of  $p\text{CO}_2$  concentration.  $n = 5$  per treatment. A significant difference between a treatment group and the control group (400 ppm for a, c and e; 14  $^{\circ}\text{C}$  for b, d and f) is indicated with an asterisk.

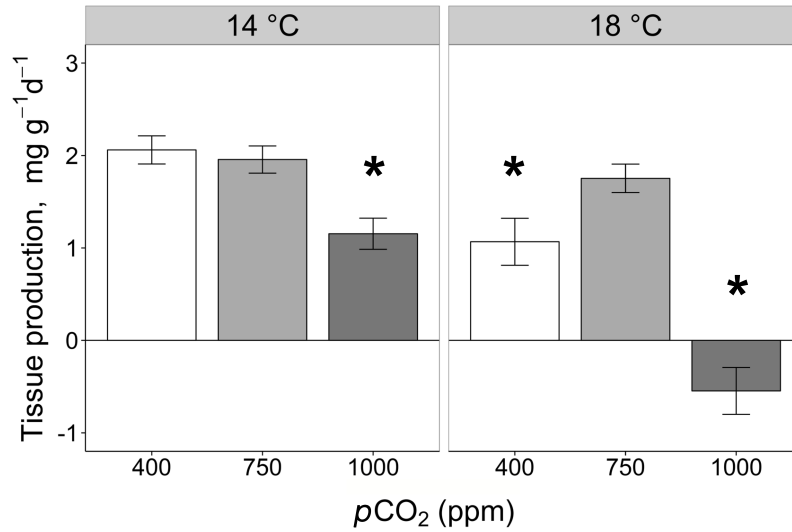


Figure 3.2: Mean ( $\pm$  S.E.) change in tissue production ( $\text{mg g}^{-1}$  (AFDW)  $\text{d}^{-1}$ ) of *N. lapillus*,  $n = 5$ . A significant difference between a treatment group and the control group (400 ppm, 14 °C) is indicated with an asterisk.

and warmer treatment, with only one individual dying in each of the other five treatments.

Linear shell extension of *N. lapillus* was not significantly affected by elevated  $p\text{CO}_2$ , temperature, or their interaction (all  $p > 0.05$ ). Net calcification of *N. lapillus* was significantly reduced by the highest  $p\text{CO}_2$  concentrations ( $F_{2,24} = 18.58$ ,  $p < 0.001$ ; Figure 3.3a), but was not significantly affected by elevated temperature ( $F_{1,24} = 0.381$ ,  $p = 0.54$ ; Figure 3.3b). Rates of oxygen uptake in *N. lapillus* were significantly elevated by both ocean acidification ( $F_{2,24} = 8.38$ ,  $p < 0.01$ ; Figure 3.3c), and temperature ( $F_{1,24} = 122.8$ ,  $p < 0.001$ ; Figure 3.3d).

### 3.3.3 Predator feeding and ingestion efficiency

Elevated  $p\text{CO}_2$  ( $F_{2,24} = 3.75$ ,  $p < 0.05$ ; Figure 3.4a) and increased temperature ( $F_{1,24} = 8.89$ ,  $p < 0.01$ ; Figure 3.4b) significantly reduced the feeding rates of *N. lapillus*, with the combination of elevated temperature and the high  $p\text{CO}_2$  concentration causing a significant reduction in consumption rates compared to all other treatments (Tukey HSD *post-hoc* test,  $p < 0.05$ ). Ingestion efficiency, the ratio of ingestion and metabolism, was significantly diminished by both ocean acidification ( $F_{2,24} = 6.34$ ,  $p < 0.01$ ; Figure 3.4c) and ocean warming ( $F_{2,24} = 19.68$ ,  $p < 0.001$ ; Figure 3.4d).

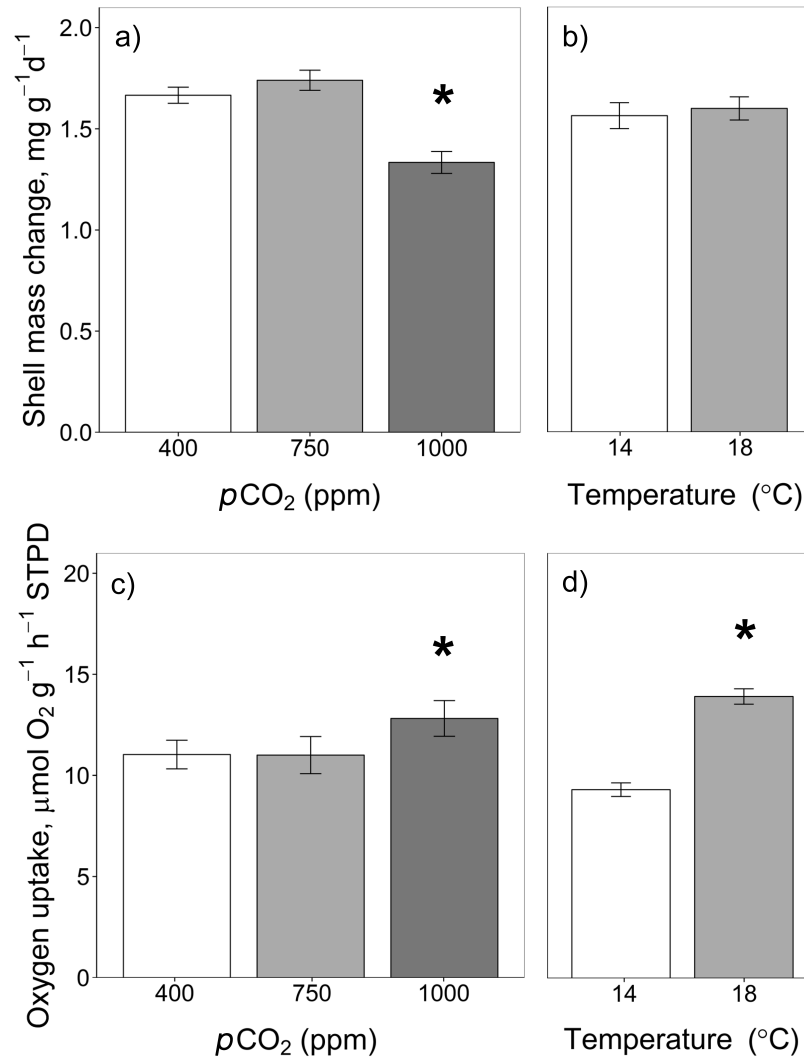


Figure 3.3: (a-b) Mean ( $\pm$  S.E.) shell mass change ( $\text{mg g}^{-1}$  (AFDW)  $\text{d}^{-1}$ ), and (c-d) Mean ( $\pm$  S.E.) metabolic rate (oxygen uptake,  $\mu\text{mol O}_2 \text{ g}^{-1}$  (AFDW)  $\text{h}^{-1}$  STPD) of *N. lapillus*. Panels a and c are in response to different  $p\text{CO}_2$  concentrations (400, 750 and 1000 ppm) averaged across levels of temperature. Panels b and d are in response to different temperature treatments (14 and 18 °C) averaged across levels of  $p\text{CO}_2$  concentration.  $n = 5$  per treatment. A significant difference between a treatment group and the control group (400 ppm for a and c; 14 °C for b and d) is indicated with an asterisk.

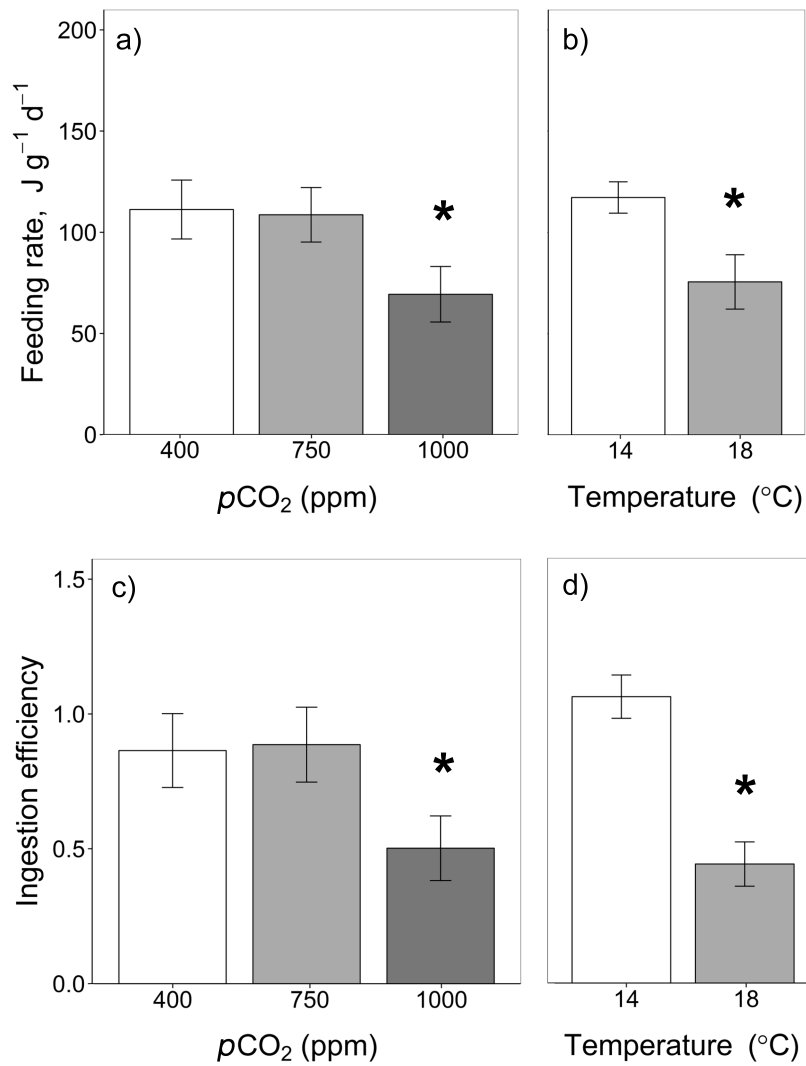


Figure 3.4: (a-b) Mean ( $\pm$  S.E) consumption rates ( $\text{J g}^{-1}$  (AFDW)  $\text{d}^{-1}$ ) and (c-d) Mean ( $\pm$  S.E) ingestion efficiency (ratio of ingestion and metabolism) of *N. lapillus* upon *S. balanoides*. Panels a and c are in response to different  $p\text{CO}_2$  concentrations (400, 750 and 1000 ppm) averaged across levels of temperature. Panels b and d are in response to different temperature treatments (14 and 18 °C) averaged across levels of  $p\text{CO}_2$  concentration.  $n = 5$  per treatment. A significant difference between a treatment group and the control group (400 ppm for a and c; 14 °C for b and d) is indicated with an asterisk.

### 3.4 DISCUSSION

Our findings suggest important changes in the energetic allocation of an intertidal predator and prey when exposed to both ocean acidification and warming. This was demonstrated through a reduction in the growth of somatic tissue for *S. balanoides*, reducing their quality as a food source. Simultaneously, the predator *N. lapillus* experienced increased energy expenditure due to higher maintenance costs, but appeared unable to initiate any compensatory mechanisms that would allow energy intake to match expenditure, and instead demonstrated reduced feeding rates and a subsequent loss of somatic tissue. Thus, future climate change may indirectly cause (and exacerbate) negative influences on the fitness of higher order consumers through bottom-up effects on basal resources.

#### 3.4.1 Prey responses

Although *S. balanoides* are capable of maintaining their calcification in response to ocean acidification (present study; Findlay *et al.*, 2009), shell maintenance is still an energetically costly process (Palmer, 1983, 1992). We found tissue production in *S. balanoides* was significantly reduced by both ocean acidification and warming, and as such suggests a reallocation of resources (e.g. use of lipid stores for energetic maintenance, Barnes *et al.*, 1963) away from somatic growth, leading to a smaller (less energetically profitable) body size in future oceans (see also Daufresne *et al.*, 2009). The warming treatment (in particular) had a prominent negative effect on the somatic growth and survival of *S. balanoides*. This is possibly due to the fact that the predicted warmer conditions will exceed those temperatures currently experienced at their (current) southern range edge in the UK (Helmuth *et al.*, 2006; Mieszkowska *et al.*, 2006). This is likely to reduce the abundance of *S. balanoides*, and have important implications for their distribution, as well as for predators that presently utilise them as a prey resource.

#### 3.4.2 Predator responses

Predation by *N. lapillus* involves alternating phases of active rasping and dissolution using chemical secretions from the accessory boring organ (Chétatl & Fournié, 1969; Carriker, 1981). The rasping rate of *N. lapillus* has been previously demonstrated to increase significantly with temperature, and warming could also theoretically increase the rate of chemical reactions used in the dissolution of the shell (Miller, 2013), thereby reducing handling times. However, we observed reduced feeding rates of *N. lapillus* when exposed to the combination of high  $p\text{CO}_2$  and warming, which may suggest that the combination of these stressors leads to limitations to consumption associated with either prey handling time, or the searching time required to find it (MacArthur & Pianka, 1966). In this study, the calcification rates of *S. balanoides* were not significantly affected, which would suggest that handling times would not be expected to change. The decreased foraging rate exhibited is therefore more likely associated with some direct effects on the predator itself. Ocean acidification has been shown to induce complex changes in chemoreception (see Nilsson *et al.*, 2012), including an impairment of the ability to detect prey (e.g. Cripps *et al.*, 2011) and it is likely that similar processes may be operating here.

The metabolic rates of *N. lapillus* were significantly stimulated by increased temperature

and to a lesser extent elevated  $p\text{CO}_2$ . This indicates an increased cost to sustain basic cellular functions and can result in energy being reallocated towards the maintenance of acid-base and metabolic disturbances, and away from vital biological processes, such as protein synthesis, growth (somatic and shell), and reproduction (e.g. [Langenbuch & Pörtner, 2002](#); [Stumpp \*et al.\*, 2012b](#); [Calosi \*et al.\*, 2013a](#)). Likely as a consequence of the increased basal metabolic demand, we found that tissue production in *N. lapillus* was reduced in response to elevated  $p\text{CO}_2$  and increased temperature, due to an altered energy reallocation ([Jansen \*et al.\*, 2007](#); [Yamane & Gilman, 2009](#); [Stumpp \*et al.\*, 2012b](#)). Moreover, when elevated  $p\text{CO}_2$  was combined with increased temperature, *N. lapillus* individuals actually exhibited somatic tissue loss akin to starvation (e.g. [Fussmann \*et al.\*, 2014](#)).

Tissue loss is commonly observed in *N. lapillus* during the non-feeding overwintering period ([Feare, 1971](#)), where body reserves are utilised as an energy source. With the majority of feeding (and growth) carried out during the summer months ([Burrows & Hughes, 1990, 1991](#)), reduced foraging success during these times may not only reduce the capacity for storing energy for later use (such as for overwintering torpor, or reproduction), but may require foraging to be maintained for extended periods of the year, increasing the risk of predation and dislodgement during storm events.

### 3.4.3 Predator-prey interactions

The core assumption of predator-prey dynamics is that consumers will be attempting to maximise their energy intake in comparison to their energy expenditure ([Gaylord \*et al.\*, 2015](#)), i.e. optimal foraging theory ([MacArthur & Pianka, 1966](#)). Here, prey quality was reduced due to elevated  $p\text{CO}_2$  and temperature, however, rather than compensating for the reduced prey quality by increasing their feeding rate (e.g. [Cruz-Rivera & Hay, 2000](#)), a reduced feeding rate was observed. Future climate change may therefore represent an important shift that is capable of not only influencing the energetic demands of all trophic levels, but also simultaneously affecting the ability of higher trophic levels to respond to changes in both resource availability and quality.

Energy gain may be constrained by food availability, digestive efficiency and digestive rate ([Calow, 1975](#)). Our observations of reduced predator foraging rates resulting in somatic tissue loss, suggesting the possibility of consumer starvation (e.g. [Fussmann \*et al.\*, 2014](#)), was reflected in diminished ingestion efficiency. This meant that as metabolic costs increased, and feeding rates concurrently decreased, the energy available for performance-related activities (e.g. growth) declined. Reduced feeding rates in response to ocean acidification and warming is a relatively common mechanism when the stressful conditions exceed an organism's tolerance (e.g. [Appelhans \*et al.\*, 2012](#); [Russell \*et al.\*, 2013](#); [Vargas \*et al.\*, 2013](#); [Miller \*et al.\*, 2014](#)). Any attenuations in the energy moving up a food chain (due to reduced feeding or prey quality) would likely result in an increased mortality rate due to starvation, and a diminished investment into reproduction; the combination of these population-level consequences could then cause a positive feedback loop that would result in the rapid non-linear decline of this species, and have fundamental consequences for community dynamics.

#### 3.4.4 *Conclusion*

In conclusion, manipulation of temperature and  $p\text{CO}_2$  to represent realistic scenarios of future climate change resulted in a reduction in the survival and somatic growth, and likely quality, of the prey. At the same time the metabolic rate of the predator increased and despite prey resources remaining abundant the consumption rates of the predator were reduced to the point that they exhibited starvation (a loss of somatic tissue). Our findings suggest that the cost of living under future climate change may surpass the energy intake from consumption rates, and that it is likely facilitated through bottom-up effects by having a reduced quality of basal resources. If responses such as observed here are more common in a warmer, high  $\text{CO}_2$  world, and given the importance of predator-prey interactions in structuring marine communities, future climate change will have major consequences for community composition and the structure and function of marine ecosystems.





## FEAR MAY BE THE LEAST OF YOUR WORRIES: ALTERED CONSUMER-RESOURCE DYNAMICS UNDER FUTURE OCEAN ACIDIFICATION AND WARMING

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### Abstract

Despite a growing understanding of species' physiological tolerances and performance in response to ocean acidification and warming, the emergent effects on the strength and direction of ecological interactions are poorly understood and yet are likely to be significant. Here, we investigated the direct and non-consumptive effects of the common shore crab *Carcinus maenas* (L., 1758) on life-history traits of two intermediate consumers (*Littorina littorea* L., 1758 and *Nucella lapillus* L., 1758) under current and future predicted  $p\text{CO}_2$  concentrations and temperature. We found that the intermediate consumers experienced increased energy demands and reduced energy intake, which limited their somatic tissue production, and suggest that they are likely to face a heightened risk of starvation in future oceans. Moreover, the predator (*C. maenas*) demonstrated an elevated feeding rate in the warmer, acidic conditions suggesting the potential for enhanced top-down control. Managing the risk of starvation in future oceans may require adopting a less risk adverse behaviour, but this will likely have to be in an environment with a greater risk of predation and increasingly adverse environmental conditions. Therefore, we propose that future climate change may represent the tipping point of the trade-off between eating and being eaten, with the behavioural choices of the organisms being hunger-driven and less balanced with safety.



## 4.1 INTRODUCTION

Humans are subjecting natural systems to rapid change through an increasing number of anthropogenic threats (Halpern *et al.*, 2007). Ocean acidification, the shift in ocean carbonate chemistry due to increased atmospheric carbon dioxide, and ocean warming represent two of the most pervasive threats to marine ecosystems (Doney *et al.*, 2009; Hoegh-Guldberg & Bruno, 2010). Despite a growing understanding of species' physiological tolerances and performance in response to climate change (including ocean acidification and warming) (Pörtner, 2012; Harvey *et al.*, 2013; Kroeker *et al.*, 2013b), the emergent effects on the strength and direction of ecological interactions are poorly understood (but see Russell *et al.*, 2013; Sanford *et al.*, 2014; Gaylord *et al.*, 2015; Harvey & Moore, *In Submission*), yet are likely to be significant. Therefore, the fate of marine biodiversity, in the face of anthropogenically induced change, cannot simply be determined by the outcome of individual species in isolation (Tylianakis *et al.*, 2008; Gilman *et al.*, 2010; Harley, 2011).

Our present understanding (certainly with regards to ocean acidification) of the outcomes of species interactions in the face of climate change in the marine environment has predominantly focussed on direct consumption in predator-prey and plant-herbivore interactions (e.g. O'Connor, 2009; Landes & Zimmer, 2012; Carr & Bruno, 2013; Russell *et al.*, 2013; Sanford *et al.*, 2014). Recent research investigating community-level responses has, however, found important alterations in the relative importance of top-down and bottom-up effects (Hoekman, 2010; Kratina *et al.*, 2012; Miller *et al.*, 2014) and indirect interactions (Barton *et al.*, 2009) in ecological communities and food webs in response to experimental warming.

Predation represents a ubiquitous ecological interaction that plays a central role in the structuring of natural communities (Paine, 1974; Menge, 1978). It is thought that predation influences ecosystem structure and functioning through two principal mechanisms: consumption, by the capture and subsequent ingestion of prey, and non-consumptive effects (hereafter, NCEs), whereby the presence of predators causes prey to exhibit phenotypic defensive responses, including changes to behaviour, life-history decisions, morphology and energy allocation (Werner & Peacor, 2003; Preisser *et al.*, 2005; Abrams, 2007; Hawlena & Schmitz, 2010). These two mechanisms have often been considered as dichotomous alternatives, whereas in reality, NCEs could either be additive or complementary with consumption (decreasing or increasing local prey abundance) (Schmitz *et al.*, 2004; Preisser *et al.*, 2005; Hawlena & Schmitz, 2010). NCEs can actually equal or exceed the impacts of direct consumption on the prey community by increasing energetic costs for the prey, causing reduced fitness (Werner & Peacor, 2003; Schmitz *et al.*, 2004). Moreover, NCEs often have additional indirect effects on other organisms in the community, such as reductions in prey activity and feeding indirectly promoting basal resources (termed trait-mediated interactions, Werner & Peacor, 2003). Consequently, predator effects on community structure and/or dynamics may be greater than would be predicted from consumption alone (Preisser *et al.*, 2005).

The strength of NCEs will be dependent on the state of their prey (Luttbeg *et al.*, 2003; Matassa & Trussell, 2014b). When there is a higher risk of predation, prey will balance the trade-off between eating and being eaten (Trussell & Schmitz, 2012). This can be achieved by reducing their foraging activities, or by utilising more refuges (Matassa &

Trussell, 2011). However, the reduced energy gain will typically result in a reduction in growth, and ultimately fitness (Trussell *et al.*, 2006; Creel *et al.*, 2007). Should environmental conditions alter the relative costs and benefits of foraging (Harvey & Moore, In Submission), then an increased risk of starvation may render this behavioural trade-off unfeasible. If that is the case, as the cost of living increases with future climate change (due to higher maintenance costs and reduced feeding, e.g. Appelhans *et al.*, 2012; Russell *et al.*, 2013; Harvey & Moore, In Submission), then prey will either need to become less risk adverse (and maintain sufficient foraging), or undergo alterations to their energy allocation strategies and deal with the population-level consequences of reduced fitness.

In order to understand the dynamics of predator-prey interactions in response to ocean acidification and warming will require understanding the physiological and ecological response of both predator and prey (Kroeker *et al.*, 2014). Therefore, we used two well-studied rocky shore predator-prey systems to test the direct and NCEs of the common shore crab *Carcinus maenas* (L., 1758) on life-history traits of the herbivorous gastropod *Littorina littorea* (L., 1758) and the carnivorous gastropod *Nucella lapillus* (L., 1758) under current and future predicted  $p\text{CO}_2$  concentrations and temperature. Predator-prey interactions already play a crucial role in structuring communities (Paine, 1974; Menge, 1978), regulating population-level demographics within the community (Leibold *et al.*, 2004), and hence understanding how predator-prey dynamics are influenced may represent the next step in scaling up the effects of ocean acidification and warming to populations, communities and ecosystems.

## 4.2 MATERIAL AND METHODS

### 4.2.1 Experimental conditions and system design

An orthogonal experimental design was employed with two levels of  $p\text{CO}_2$  (400 ppm, 1000 ppm), two levels of temperature (14 °C and 18 °C), and two levels of predation risk (- Risk, + Risk). Levels of temperature and  $p\text{CO}_2$  were chosen based on the IPCC [2007b] A1F1 scenario for the year 2100. This scenario is mostly comparable to the IPCC [2013] RCP 8.5 scenario (Rogelj *et al.*, 2012). Predation risk was achieved by placing a single male crab (*C. maenas*, carapace width 40-50 mm) behind a perforated barrier within every experimental tank in the + Risk treatment, along with five *N. lapillus* or *L. littorea* (relevant to the species being tested) that served as food for the crab. This ensured both alarm cues from conspecifics, and effluent from crushed conspecifics (Dalesman *et al.*, 2006), thus providing the prey with the olfactory cues of predator kairomones and prey death pheromones to ensure a maximal anti-predator response (Trussell & Nicklin, 2002; Cotton *et al.*, 2004), while preventing any physical contact between crabs and the experimental snails. No-predator cue treatments also had five *N. lapillus* or *L. littorea* as controls, which were also placed behind the perforated barrier, being replaced (in both cases) every 5 days.

The experimental system was in a constant temperature room with aquarium heaters (Eheim Jäger 300W) used in the footer tank to maintain elevated temperature treatments. The pH of the mesocosms was set by continuous bubbling of a known air- $\text{CO}_2$  mix (LiCOR IRGA LI-820, Lincoln, NE USA).  $\text{pH}_{\text{NBS}}$  (Mettler-Toledo SevenGo Pro pH meter with Inlab 413SG probe, Mettler-Toledo, Germany), temperature and salinity (WTW Cond 3210, WTW, Germany) were recorded daily, and total alkalinity ( $A_T$ , acid-base titration)

was measured weekly. In order to calculate the additional carbonate chemistry parameters, following Nisumaa *et al.* [2010], the dissolved inorganic carbon ( $C_T$ ) was calculated using the software CO2SYS (Lewis & Wallace, 1998), with the measured  $pH_{NBS}$  and  $A_T$  as the input variables. Subsequently, the additional carbonate system parameters were calculated using the R package seacarb (Lavigne & Gattuso, 2010) using the calculated  $C_T$  and measured  $A_T$  (Table 4.1). For both CO2SYS and seacarb, we used dissociation constants from Mehrbach *et al.* [1973], as adjusted by Dickson & Millero [1987], and  $KSO_4$  using Dickson [1990] (Table 4.1). Light conditions were adjusted weekly to maintain natural light:dark cycles, seawater was replenished weekly, and fresh water added (every two to three days) to account for any salinity increases due to evaporation.

#### 4.2.2 Animal collection and experimental design

The experimental snails, juvenile *N. lapillus* (shell length 10-15 mm) and *L. littorea* (shell length 8-10 mm), and male *C. maenas* (carapace width 40-50 mm) were collected from Aberystwyth, Wales, UK (52° 24' 54.9" N, 4° 05' 28.7" W) during July 2013. Following collection, all three species were maintained (separately) in a flow-through aquarium system for three days. These species were chosen as they represent model, ubiquitous, temperate rocky-shore species.

Two experiments were carried out. The first experiment maintained *N. lapillus* and *L. littorea* (separately) under experimental conditions in tanks (225 × 165 × 78 mm) for 60 days (four individuals per tank,  $n = 6$  tanks). Throughout this experiment, *N. lapillus* were fed *Mytilus edulis* (L., 1758) and *L. littorea* were fed *Fucus serratus* (L., 1753) *ad libitum*. Although fucoids are not the preferred food source for *L. littorea*, their preferred food source of ephemeral green algae (Lubchenco, 1978) is not suitable for the longer-term experimental conditions.

The second experiment was a feeding trial, using individuals of *N. lapillus* and *L. littorea* taken from the first experiment (and fed in the same manner). This was carried out over 14 days as paired replicates ( $n = 12$  for *N. lapillus*,  $n = 6$  for *L. littorea*) with the experimental snails either present (one individual) or absent (control). During the feeding trials, the experimental snails were placed within smaller pots (75 (d) × 35 mm, located within the experimental tanks) in order to collect any egested food. Finally, those experimental snails used for feeding trials were maintained for a further 10 days in order to allow the gut passage time required to collect any further egested food (Grahame, 1973).

#### 4.2.3 Responses of the predator

During the first experiment, the number of *N. lapillus* and *L. littorea* consumed by *C. maenas* was recorded every five days in the + Risk treatments (i.e. the number of individuals that were supplied as food for the crab, and not the experimental individuals). Every five days, any consumed individuals were replaced to maintain the density of the 'food supply' at five individuals (from a holding tank held at the same experimental conditions). At the same time, any individuals remaining after the five days were also swapped (with individuals from the holding tank). This was to ensure that the 'food supply' was not starving over the duration of the experimental period (i.e. 60 days), since *N. lapillus* in the holding

Table 4.1: Seawater properties during experiment one.  $\text{pH}_T$ , temperature, salinity, and total alkalinity ( $A_T$ ) are measured values. Seawater  $\text{pCO}_2$ , dissolved inorganic carbon ( $C_T$ ), bicarbonate ( $\text{HCO}_3^-$ ), carbonate ( $\text{CO}_3^{2-}$ ), carbon dioxide ( $\text{CO}_2$ ), saturation states for calcite ( $\Omega_{\text{calcite}}$ ) and aragonite ( $\Omega_{\text{aragonite}}$ ) are values calculated using the carbonate chemistry system analysis program CO2Calc and the R package seacarb. Numbers in parentheses represent S.E.

Target:	$\text{pH}_T$	Temp °C	Salinity psu	$A_T$ $\mu\text{mol kg}^{-1}$	$\text{pCO}_2$ $\mu\text{atm}$	$C_T$ $\mu\text{mol kg}^{-1}$	$\text{HCO}_3^-$ $\mu\text{mol kg}^{-1}$	$\text{CO}_3^{2-}$ $\mu\text{mol kg}^{-1}$	$\text{CO}_2$ $\mu\text{mol kg}^{-1}$	$\Omega_{\text{calc}}$	$\Omega_{\text{arag}}$
400ppm +0 °C	8.041 (0.003)	14.01 (0.03)	34.63 (0.716)	2542 (44.14)	454.2 (3.835)	2321.9 (1.767)	2139.8 (2.728)	164.5 (1.122)	17.6 (0.161)	3.943 (0.027)	2.523 (0.017)
1000ppm +0 °C	7.737 (0.001)	14 (0.01)	34.64 (2.515)	2588.9 (31.67)	1011.3 (2.768)	2498 (0.432)	2368.4 (0.54)	90.4 (0.222)	39.1 (0.114)	2.168 (0.005)	1.387 (0.003)
400ppm +4 °C	8.039 (0.001)	18 (0.01)	35.42 (0.54)	2534 (42.66)	455.6 (1.438)	2282.9 (0.619)	2081.4 (0.973)	185.9 (0.4)	15.7 (0.047)	4.468 (0.01)	2.884 (0.006)
1000ppm +4 °C	7.729 (0.001)	18.01 (0.03)	35.72 (0.81)	2540.1 (34.12)	1025.8 (1.484)	2431.5 (0.396)	2295.9 (0.538)	100.3 (0.224)	35.2 (0.082)	2.411 (0.005)	1.557 (0.004)

tank could be fed with *M. edulis*, whereas any mussels placed in the experimental tanks would likely be consumed by the crab instead, confounding estimates of feeding rates. In order to account for natural mortality in the consumption rates of *C. maenas*, mortality rates in the control 'food supply' i.e. the - Risk treatment, were also recorded.

#### 4.2.4 Consumer growth, induced defences and tissue production

Before the first experiment, each experimental *N. lapillus* and *L. littorea* (four individuals per tank) was labelled with a numbered, coloured queen bee tag (EH Thorne Ltd, Rand, UK) and measured for dry shell mass and wet body mass using the non-destructive methodology of Palmer [1982]. These measurements were repeated at the end of the experiment, and (individual) dry shell growth (i.e. net calcification) and wet body growth were calculated as final mass - initial mass. Dry shell mass was calculated using linear regression with buoyant mass; for *N. lapillus* this was previously established in Chapter 3 (dry shell mass =  $1.6317 \times \text{buoyant mass} - 0.0949 \text{ g}$ ,  $n = 50$ ,  $R^2 = 0.9895$ ,  $p < 0.001$ ), and for *L. littorea* this was previously calculated by destructively sampling individuals collected at the same time and place as the experimental individuals (dry shell mass =  $1.544 \times \text{buoyant mass} + 0.0173 \text{ g}$ ,  $n = 50$ ,  $R^2 = 0.9988$ ,  $p < 0.001$ ). Wet body mass was calculated by subtracting the estimated dry shell mass from the total mass of the individual in air. Tissue production was estimated by converting the (initial and final) wet body mass to dry body mass with linear regression, for *N. lapillus* (dry body mass =  $0.2142 \times \text{wet body mass} - 0.006 \text{ g}$ ,  $n = 50$ ,  $R^2 = 0.9870$ ,  $p < 0.001$ ; this regression was established in Chapter 3), and for *L. littorea* (dry body mass =  $0.1914 \times \text{wet body mass} - 0.042 \text{ g}$ ,  $n = 50$ ,  $R^2 = 0.9661$ ,  $p < 0.001$ ; this regression was established with the same individuals that were used for the dry shell mass to buoyant mass relationship).

#### 4.2.5 Consumer metabolism, feeding rates and assimilation efficiency

Oxygen uptake, as a proxy for metabolic rate, was measured using closed bottle respirometers following the methodology used in Chapter 3. Individual gastropods were placed in blacked out 250 ml respirometers (*N. lapillus*,  $n = 10$  and *L. littorea*,  $n = 18$ ) placed in a water bath to maintain their respective temperature,  $p\text{CO}_2$  treatments and predation risk treatments. Predation risk treatments were achieved by placing two male *C. maenas* (carapace width 40-50 mm) into the water bath 24 hours prior to the determination of metabolic rate, along with five gastropods (of the same species as the experimental snails) as a food source. Five additional blanks (per treatment) were tested at the same time to account for microbial respiration. Respirometers were left open (but covered) for one hour prior to measurements to remove any potential handling stress. Rates of oxygen uptake were taken as the difference in dissolved oxygen between the starting measurement (immediately before sealing the respirometers) and the final measurement (taken after the respirometers were sealed for 1 hour). Measurements were then multiplied by the solubility coefficient for oxygen (corrected for temperature and salinity, Harvey, 1955) and the respirometer volume, for standard temperature and (dry) pressure (STPD). Ash-free dry body mass was measured by sacrificing all individuals measured for their metabolic rate (6 hrs at 500 °C), and final oxygen uptake was expressed in terms of  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ (AFDW)} \text{ h}^{-1} \text{ STPD}$ .

For *N. lapillus*, feeding rates were measured by the amount of *M. edulis* tissue mass



consumed. Ten *M. edulis* (shell length 10-18 mm) were used for each feeding trial ( $n = 12$ ), and feeding rates were measured by the number of empty, drilled *M. edulis* when *N. lapillus* were present. Each consumed *M. edulis* individual was measured for shell length (digital callipers,  $\pm 0.1$  mm) and an ash-free dry mass was calculated using an allometric regression with shell length (previously calculated by destructively sampling individuals collected at the same time and place as the experimental individuals; ash-free dry tissue mass =  $0.0326 \times \text{shell length}^{2.7164}$  mg,  $n = 50$ ). Deceased *M. edulis* were replaced daily with individuals from a holding tank (under the same experimental conditions), so that ten prey items were always available to *N. lapillus*. At the completion of the feeding trials, any half-eaten mussels were removed and the ash-free dry tissue mass of the remnants measured (500 °C, 6 h). This ash-free dry mass of the remaining food was then taken from the estimated (whole mussel) ash-free dry tissue mass, and incorporated as part of total feeding. Autogenic controls ( $n = 12$ ) were used to account for any natural mortality of *M. edulis* (i.e. in the absence of *N. lapillus*). The remaining living mussels were measured for shell length (digital callipers,  $\pm 0.1$  mm) and ash-free dry tissue mass (500 °C, 6 h), in order to establish a new allometric relationship to test whether the effects of  $p\text{CO}_2$ , temperature or predation risk influenced the length-mass relationship of *M. edulis*. The per capita amount of energy acquired was estimated using the calorific values of  $19.5 \text{ J mg}^{-1}$  of *M. edulis* tissue (Elnor & Hughes, 1978).

For *L. littorea*, feeding rates were measured by the amount of *F. serratus* consumed during the feeding trials. Initial (blotted) wet mass of *F. serratus* was  $1.29 \pm 0.03 \text{ g}$  (S.E.). Wet mass was converted to dry mass using a dry:wet ratio (dry mass =  $0.261 \times \text{wet mass} + 10.07 \text{ mg}$ ,  $n = 36$ ,  $p < 0.001$ ) which was established using *F. serratus* collected at the same time as those used for the feeding trials. The quantity of *F. serratus* consumed was quantified as final dry mass (60 °C, 24 h) - initial dry mass where *L. littorea* was present ( $n = 6$ ), using the controls (*F. serratus* in the absence of *L. littorea*) to account for autogenic loss ( $n = 6$ ). The remaining *F. serratus* were measured for dry mass (60 °C, 24 h), in order to test whether the effects of  $p\text{CO}_2$ , temperature or predation risk influenced the autogenic loss of *F. serratus*. The per capita amount of energy acquired was estimated using the calorific values of  $14.35 \text{ J mg}^{-1}$  dry mass (based on *Fucus distichus*, Paine & Vadas, 1969).

For both *L. littorea* and *N. lapillus*, we measured the gravimetric assimilation efficiency (%) as  $((\text{ingested food} - \text{egested food}) \times \text{ingested food}) \times 100$ . Egested food was measured as faecal material from each experimental snail during the 24-day period (two week feeding trial and subsequent 10 days for gut passage clearing). Egested food was rinsed in deionised water, filtered using a vacuum pump (Fisherbrand, Fisher Scientific) and (pre-weighed) Whatman no. 1 filter papers (110 mm) and finally, dry mass was obtained (60 °C).

#### 4.2.6 Effect size interactions

We used the interaction effect size (Hedge's  $g$ , Hedges & Olkin, 1985) to classify the effect that combinations of drivers (elevated  $p\text{CO}_2$ , increased temperature, predation cues) exhibit when interacting, following the methodology of Gurevitch *et al.* [2000]. For exam-



ple, the interaction effect size and associated variance between elevated  $p\text{CO}_2$  (OA) and temperature (T) were calculated as follows:

$$g_{int} = \frac{(\bar{y}_{OA+T} - \bar{y}_T) - (\bar{y}_{OA} - \bar{y}_{Control})}{S_{pooled}} J(m) \quad (4.1)$$

Where,  $\bar{y}$  is the mean response,  $s$  is the pooled standard deviation, and  $J(m)$  is the small sample size bias correction.

$$S_{pooled} = \sqrt{\frac{(N_{Control} - 1)(S_{Control})^2 + (N_{OA} - 1)(S_{OA})^2 + (N_T - 1)(S_T)^2 + (N_{OA+T} - 1)(S_{OA+T})^2}{N_{Control} + N_{OA} + N_T + N_{OA+T} - 4}} \quad (4.2)$$

where  $N$  is the sample size and  $s_i$  is the standard deviation of the mean at each treatment level. Finally, the variance of  $g$  for the interaction is:

$$s^2 g_{int} = \frac{1}{N_{Control}} + \frac{1}{N_{OA}} + \frac{1}{N_T} + \frac{1}{N_{OA+T}} + \frac{g_i^2}{2(N_{Control} + N_{OA} + N_T + N_{OA+T})} \quad (4.3)$$

Interaction effect sizes ( $\pm 95\%$  CI) that overlapped zero were classified as additive (no additional effect), effects sizes of  $>0$  were antagonistic (reduced effect) and effects sizes of  $<0$  were classified synergistic (enhanced effect). This assumed that the individual effect sizes were either all negative, or a combination of both negative and positive values. If individual effect sizes were all positive, then the opposite interpretation was assumed. A positive value for the interaction effect size shows that (for example) ocean acidification has more effect when warming was occurring concurrently than in isolation. For the three-way interaction (1000 ppm  $p\text{CO}_2$ ,  $18^\circ\text{C}$  temperature, and the presence of predation risk), we only tested the two-way interaction between the combination of 1000 ppm  $p\text{CO}_2$  and  $18^\circ\text{C}$  (together), with the presence and absence of risk (shown in equations 4.4-4.6).

$$g_{int} = \frac{(\bar{y}_{OA+T+Risk} - \bar{y}_{OA+T}) - (\bar{y}_{Risk} - \bar{y}_{Control})}{S_{pooled}} J(m) \quad (4.4)$$

$$S_{pooled} = \sqrt{\frac{(N_{Control} - 1)(S_{Control})^2 + (N_{Risk} - 1)(S_{Risk})^2 + (N_{OA+T} - 1)(S_{OA+T})^2 + (N_{OA+T+Risk} - 1)(S_{OA+T+Risk})^2}{N_{ct} + N_{acid} + N_{temp} + N_{both} - 4}} \quad (4.5)$$

$$s^2 g_{int} = \frac{1}{N_{Control}} + \frac{1}{N_{Risk}} + \frac{1}{N_{OA+T}} + \frac{1}{N_{OA+T+Risk}} + \frac{g_i^2}{2(N_{Control} + N_{Risk} + N_{OA+T} + N_{OA+T+Risk})} \quad (4.6)$$

This approach was applied because ocean acidification and warming are anticipated to occur concurrently, and this study aimed to specifically test the effect that the addition of predation risks would have on the combined stressors, relative to risk in the control conditions. See Figure C.1 for the interaction effect size for each response variable.

#### 4.2.7 Statistical analysis

Statistical analyses were conducted using R (R Development Core Team, 2012). In all cases data were normally distributed (Kolmogorov-Smirnov) as well as displaying homogeneity of variance (Levene) ( $p > 0.05$ ). Three-way ANOVA was employed to test for possible differences among all measurements between treatments, with Tukey HSD *post-hoc*

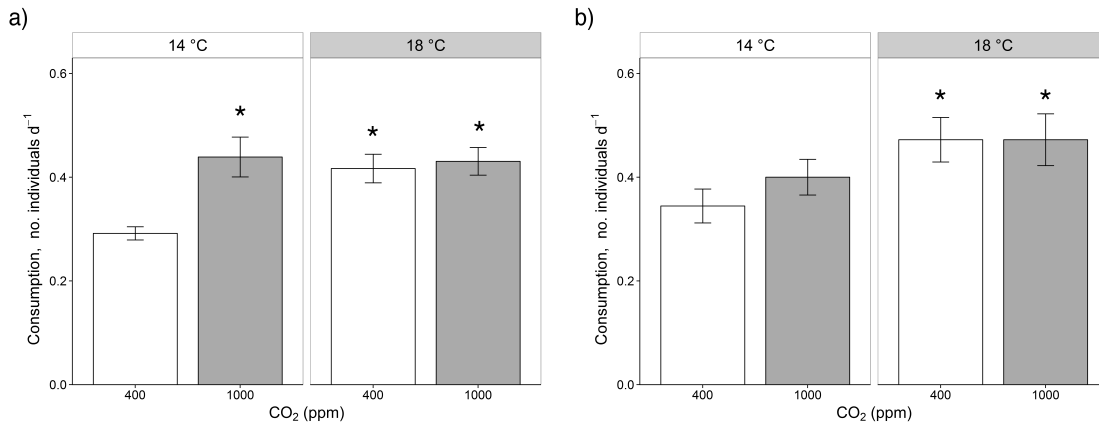


Figure 4.1: Mean ( $\pm$  S.E.) consumption by *C. maenas* (individuals d<sup>-1</sup>), of a) *N. lapillus*, and b) *L. littorea*. Significant differences from control conditions (400 ppm and 14 °C) are indicated by an asterisk.

tests used to test pair-wise differences. An ANCOVA was employed to test whether any treatments ( $p\text{CO}_2$ , temperature and predation risk) influenced the relationship between *M. edulis* ash-free dry tissue mass using shell length as a covariate, and compared to the initial length-mass relationship. Both shell length and ash-free dry tissue mass were log-transformed.

### 4.3 RESULTS

#### 4.3.1 Responses of the predator

During the first experiment, the number of *N. lapillus* consumed by *C. maenas* showed a significant interaction between elevated  $p\text{CO}_2$  concentrations and temperature ( $F_{1,20} = 5.72$ ,  $p < 0.05$ ; Figure 4.1a), whereby all three treatments ( $p\text{CO}_2$ , temperature and combined) demonstrated elevated consumption rates relative to the control conditions. The number of *L. littorea* consumed by *C. maenas* was significantly elevated by temperature ( $F_{1,20} = 6.07$ ,  $p < 0.05$ ; Figure 4.1b), but was not affected by either elevated  $p\text{CO}_2$  conditions or their interaction (both  $p = 0.50$ ; Figure 4.1b).

#### 4.3.2 Responses of the prey resources

In the feeding trials for *N. lapillus*, the prey quality of *M. edulis* (based on somatic tissue mass) significantly varied with shell length ( $F_{1,471} = 3234$ ,  $p < 0.001$ ), and was negatively affected by the presence of predation risk ( $F_{1,471} = 78.95$ ,  $p < 0.001$ ) based on regressions of log-transformed somatic tissue mass against log-transformed shell length of the live *M. edulis* in the autogenic control tanks of the feeding trial (i.e. with no *N. lapillus* present). The (same) length-mass relationship was not significantly affected by elevated  $p\text{CO}_2$  conditions ( $F_{1,471} = 0.1$ ,  $p = 0.75$ ), increased temperature ( $F_{1,471} = 2.59$ ,  $p = 0.11$ ), or any interaction between  $p\text{CO}_2$ , temperature and predation risk (pairwise interactions all  $p > 0.66$ , three-way interaction  $p = 0.23$ ). During the feeding trials for *L. littorea*, autogenic loss of *F. serratus* (in the paired replicate without *L. littorea*) was not significantly affected by elevated  $p\text{CO}_2$  conditions ( $F_{1,40} = 1.40$ ,  $p = 0.24$ ), or predation

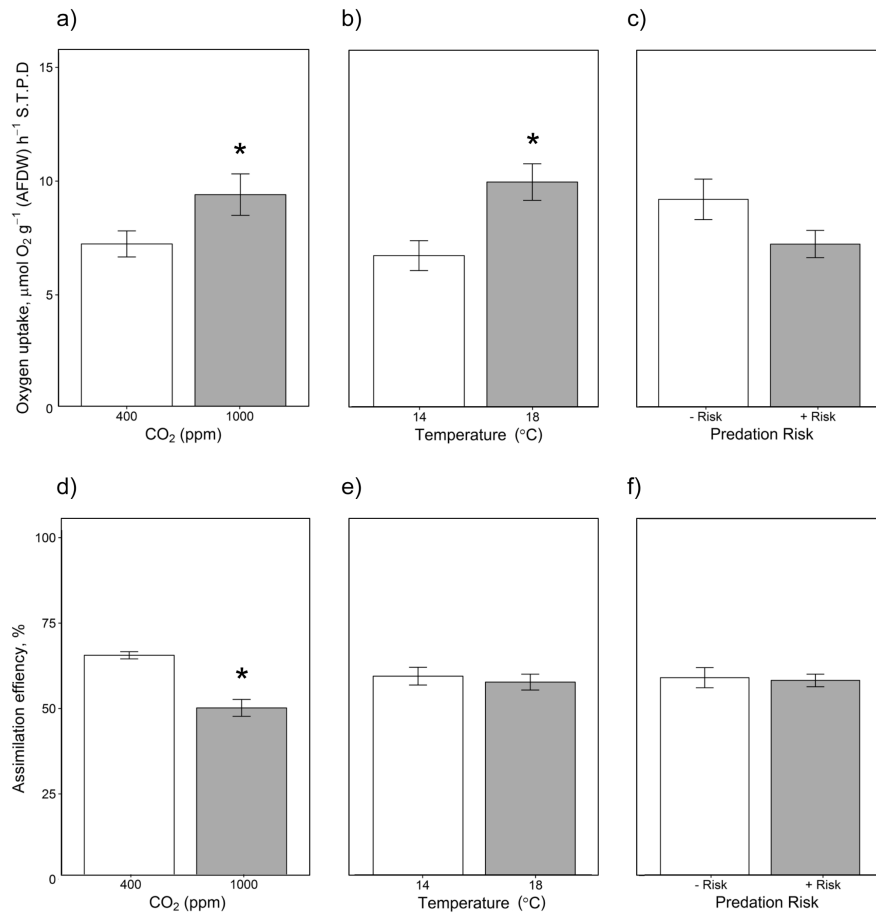


Figure 4.2: Mean ( $\pm$  S.E.) (a-c) metabolic rate (oxygen uptake,  $\mu\text{mol O}_2 \text{ g}^{-1} (\text{AFDW}) \text{ h}^{-1} \text{ STPD}$ ),  $n = 10$ , and (d-f) assimilation efficiency (%),  $n = 12$ , of *N. lapillus*. Panels a and c are in response to different  $p\text{CO}_2$  concentrations (400 and 1000 ppm) averaged across levels of temperature and predation risk. Panels b and e are in response to different temperature treatments (14 and 18 °C) averaged across levels of  $p\text{CO}_2$  concentration and predation risk. Panels c and f are in response to the absence (- Risk) and presence (+ Risk) of predation risk averaged across levels of  $p\text{CO}_2$  concentration and temperature. Significant differences are indicated by an asterisk, see Table 4.2 for more detailed statistics.

risk ( $F_{1,40} = 0.71$ ,  $p = 0.41$ ). Elevated temperature had a marginally non-significant effect on increasing the autogenic loss of *F. serratus* ( $F_{1,40} = 3.97$ ,  $p = 0.054$ ).

#### 4.3.3 *N. lapillus* metabolism, assimilation efficiency and energetic intake

The oxygen uptake responses of *N. lapillus* were significantly increased by elevated  $p\text{CO}_2$  concentrations and increased temperature (Table 4.2; Figure 4.2a-b). The presence of predation cues reduced oxygen uptake rates, but this was marginally non-significant (Table 4.2; Figure 4.2c). The mean assimilation efficiency of *N. lapillus* was significantly reduced from  $65.57 \pm 1.05$  % (S.E.) to  $50.2 \pm 2.46$  % (S.E.) in response to elevated  $p\text{CO}_2$ , but was not significantly influenced by either predation risk or warming (Table 4.2; Figure 4.2d-f).

Table 4.2: Three factor ANOVA on the effects of  $p\text{CO}_2$  (400 ppm and 1000 ppm), temperature (14 °C and 18 °C) and predation risk (- Risk, + Risk), on metabolic rate, energy intake and energy allocation of *N. lapillus*. For factors, degrees of freedom (*df*), *F*-values and significance levels are provided, with *df* and mean square (MS) values provided for the residuals. Significant tests are shown in bold. \*\*\*  $p < 0.001$ ; \*\*  $0.001 < p < 0.01$ ; \*  $0.01 < p \leq 0.05$ . Results for Tukey HSD *post-hoc* tests are presented below the table.

Factor	Metabolic rate		Energy intake			Energy allocation		
	<i>df</i>	Oxygen uptake	<i>df</i>	Feeding	Assimilation	Intake	<i>df</i>	Calcification
$p\text{CO}_2$ (OA)	1	<b>4.49 *</b>	1	<b>70.59 ***</b>	<b>31.93 ***</b>	<b>278.46 ***</b>	1	<b>32.91 ***</b>
Temperature (T)	1	<b>10.04 **</b>	1	0.14	0.41	3.08	1	0.19
Cue (C)	1	3.74	1	<b>64.24 ***</b>	0.09	<b>72.42 ***</b>	1	<b>9.53 **</b>
OA $\times$ T	1	0.01	1	<b>9.7 **</b>	3.04	<b>24.32 ***</b>	1	<b>7.03 *</b>
OA $\times$ C	1	0	1	0.13	0.07	0.04	1	0.02
T $\times$ C	1	0.37	1	1.56	0.2	0.11	1	0
OA $\times$ T $\times$ C	1	0.17	1	2.82	0.49	<b>3.85 *</b>	1	0.27
Residuals (MS)	72	20.93	88	5.72	0.01	776.96	40	1.40
Tukey's <i>post-hoc</i> tests:								
$p\text{CO}_2$ (OA)	1000>400			400>1000	400>1000	400>1000		400>1000
Temperature (T)	18>14			–	–	18>14		–
Cue (C)	– Risk > + Risk			– Risk > + Risk	–	– Risk > + Risk		– Risk > + Risk
OA $\times$ T	–			400:18 > 400:14 1000:14 = 1000:18	–	400:18 > 400:14 1000:14 = 1000:18 nb. 400:14 = 1000:14		–
T $\times$ C	–			–	–	–		18: – Risk = 14: – Risk = 14: + Risk 18: – Risk > 18: + Risk 14: – Risk > 18: + Risk
OA $\times$ T $\times$ C				–	–	400:18 > 400:14 > 1000:14 = 1000:18   nb. – Risk > + Risk, for like-for like comparisons		–

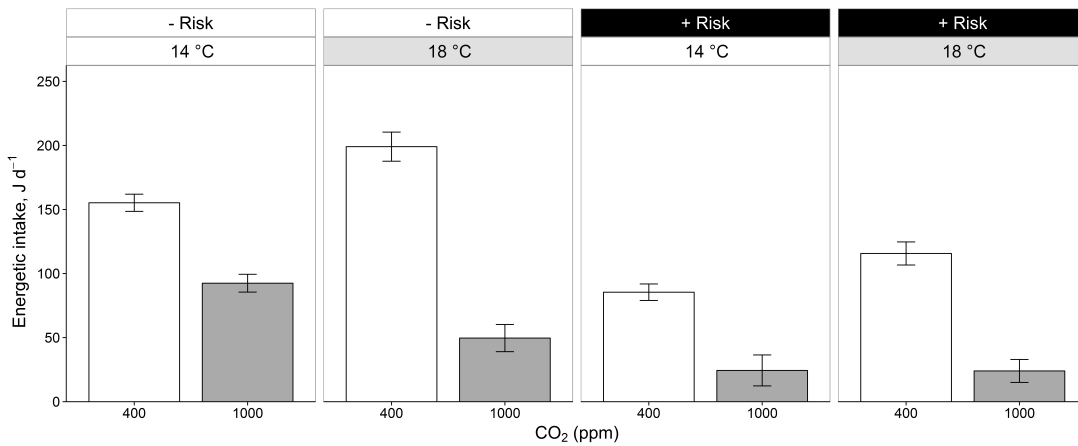


Figure 4.3: Mean ( $\pm$  S.E.) energetic intake ( $\text{J d}^{-1}$ ) of *N. lapillus*,  $n = 12$ . See Table 4.2 for significant differences.

Energetic intake responses represent the combination of feeding rates and assimilation efficiency. Since (for the most part) the patterns of energetic intake mirror those of feeding rates, only the energetic intake responses will be described for *N. lapillus*. The only exception is for elevated  $p\text{CO}_2$  treatments where reduced assimilation efficiency (Figure 4.2d) meant that feeding rates differed from energetic intake. The feeding rates are, however, presented in the Appendix (Figure C.2), and the statistics for both energetic intake and feeding rates are presented in Table 4.2.

Energetic intake demonstrated a significant three-way interaction in response to  $p\text{CO}_2$  concentrations, temperature and predation risk (Table 4.2; Figure 4.3). In order to tease this apart, we investigated the energetic intake responses, to the  $p\text{CO}_2$  and temperature treatments, in the absence and presence of predation risk. In the absence of predation risk, a significant interaction was observed between  $p\text{CO}_2$  concentrations and temperature ( $F_{1,44} = 79.14$ ,  $p < 0.001$ ; Figure 4.3). This was due to increased temperature having a positive effect on energy intake in control  $p\text{CO}_2$  conditions, but no significant effect at elevated  $p\text{CO}_2$  conditions (Table 4.2). When predation risks were present, a similar interaction between  $p\text{CO}_2$  concentrations and temperature was observed ( $F_{1,44} = 14.71$ ,  $p < 0.001$ ). Overall, the addition of predation cues reduced the energetic intake of *N. lapillus*, and when considered in terms of the three-way interactions, the presence of predation risks significantly reduced energetic intake in a like-for-like comparison (i.e. '400 ppm, 14 °C, - Risk' compared to '400 ppm, 14 °C, + Risk' etc., see Table 4.2; Figure 4.3).

#### 4.3.4 *N. lapillus* tissue production and shell mass change

Tissue production in *N. lapillus* showed a significant interaction between  $p\text{CO}_2$  concentrations and temperature (Table 4.2), due to elevated  $p\text{CO}_2$  conditions only having a significant negative effect in the elevated temperature treatment (Table 4.2; Figure 4.4a). Predation risk had an overall significant negative effect on somatic growth, but did not significantly interact with either  $p\text{CO}_2$  concentrations or temperature (Table 4.2; Figure 4.4a). Changes in shell mass were most strongly affected by ocean acidification, with increases in  $p\text{CO}_2$  concentrations causing a large reduction in the growth of shell mass (Table 4.2; Figure 4.4b). Generally, elevated  $p\text{CO}_2$  conditions had the tendency to negate

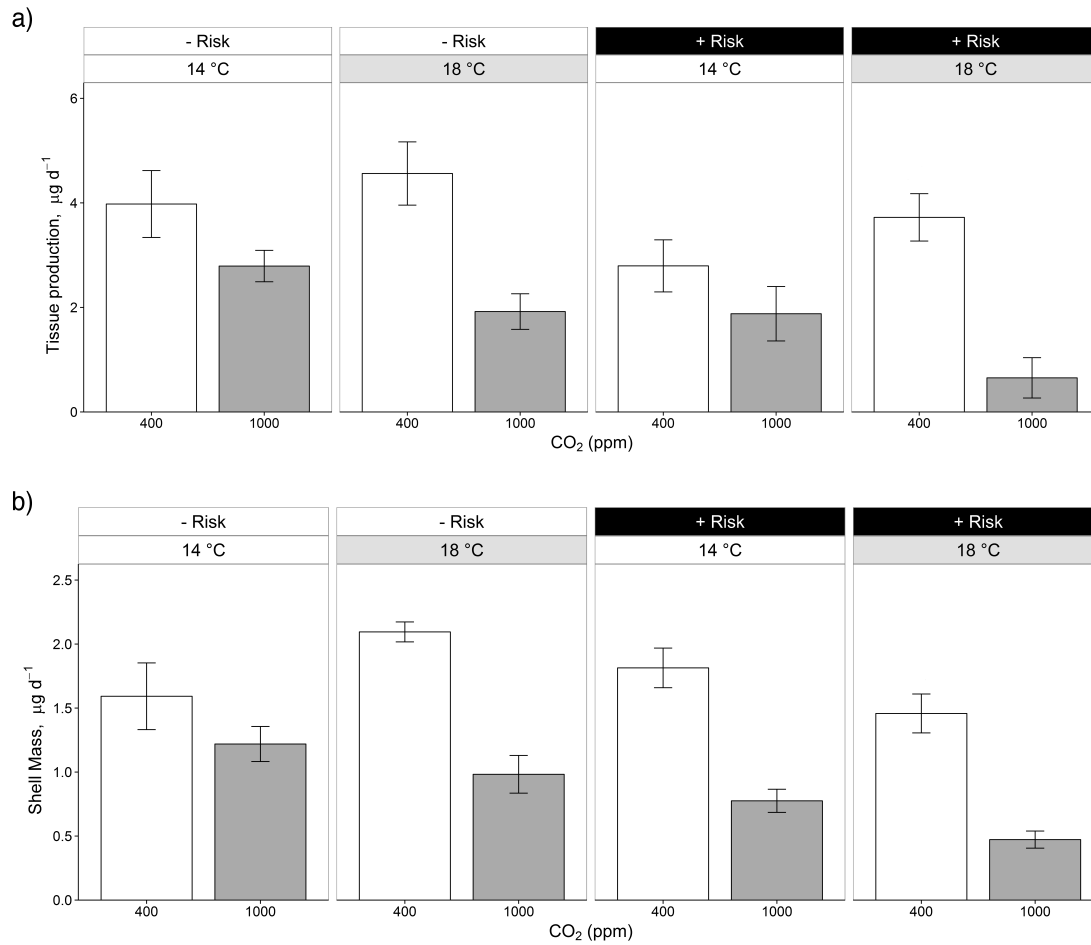


Figure 4.4: Mean ( $\pm$  S.E.) a) tissue production ( $\mu\text{g d}^{-1}$ ), b) change in length ( $\mu\text{m d}^{-1}$ ), and c) shell mass ( $\mu\text{g d}^{-1}$ ) of *N. lapillus*,  $n = 6$ . See Table 4.2 for significant differences.

or reverse any effects of temperature and predation risk towards a negative response. We also observed a significant interaction between temperature and predation cues for shell mass change (Table 4.2). This was due to the presence of predation risks reversing the direction of the response from a positive trend (in the absence of predation risk) to a negative response when both increased temperature and predation cues were combined (Table 4.2; Figure 4.4b).

#### 4.3.5 *L. littorea* metabolism, assimilation efficiency, feeding and energetic intake

Oxygen uptake rates of *L. littorea* were significantly elevated by increased temperature, but were not significantly affected by either elevated  $p\text{CO}_2$  concentrations or predation risk (Table 4.3; Figure 4.5a-c). The mean assimilation efficiency of *L. littorea* was significantly reduced from  $60.4 \pm 2.05$  % (S.E.) to  $45.1 \pm 1.38$  % (S.E.) due to elevated  $p\text{CO}_2$  concentrations, but was not significantly influenced by either warming or predation risk (Table 4.3; Figure 4.5d-f).

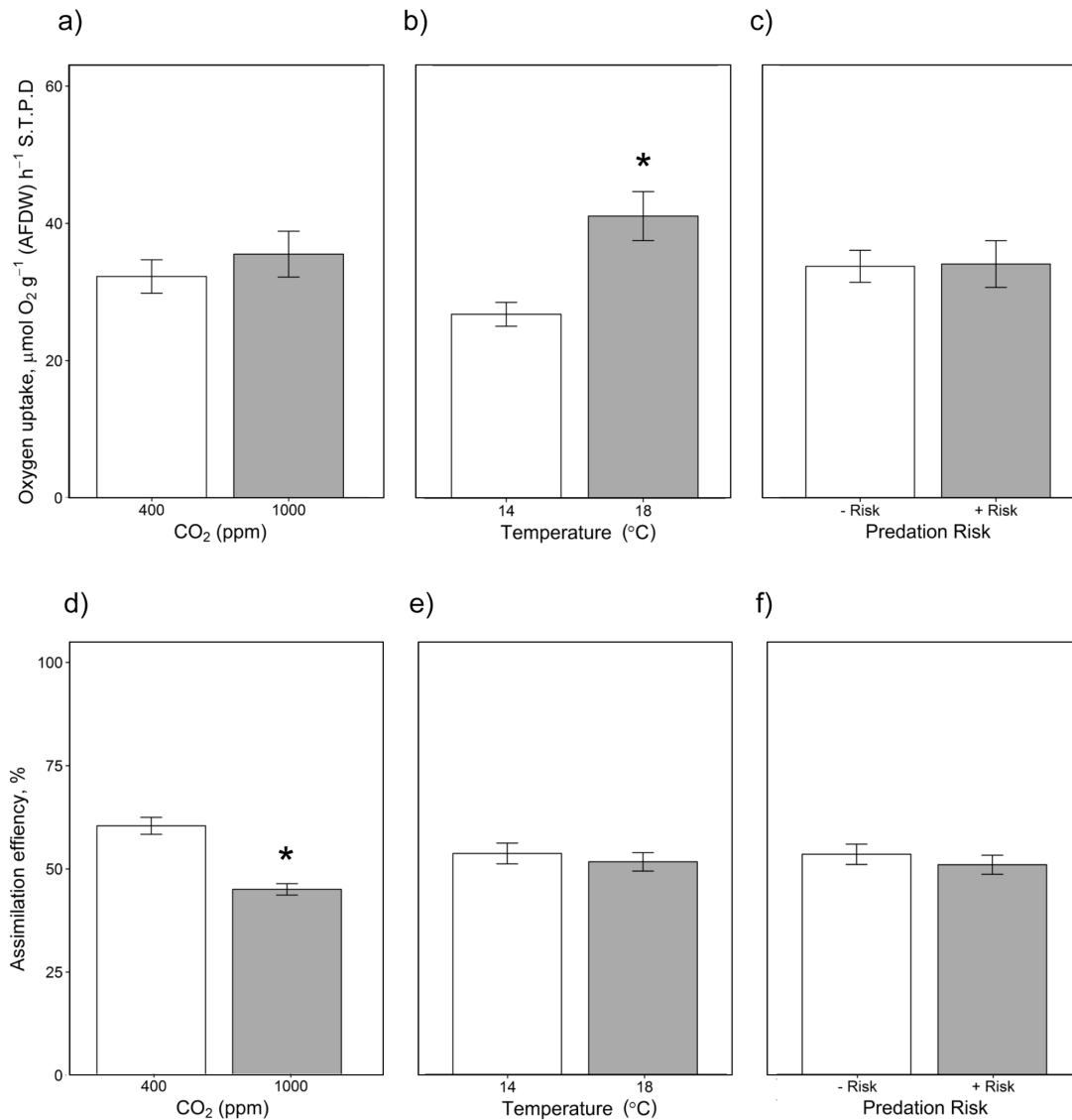


Figure 4.5: Mean ( $\pm$  S.E.) (a-c) metabolic rate (oxygen uptake,  $\mu\text{mol O}_2 \text{ g}^{-1} (\text{AFDW}) \text{ h}^{-1} \text{ STPD}$ ),  $n = 18$ , and (d-f) assimilation efficiency (%),  $n = 6$ , of *L. littorea*. Panels a and d are in response to different  $p\text{CO}_2$  concentrations (400 and 1000 ppm) averaged across levels of temperature and predation risk. Panels b and e are in response to different temperature treatments (14 and 18  $^{\circ}\text{C}$ ) averaged across levels of  $p\text{CO}_2$  concentration and predation risk. Panels c and f are in response to the absence (- Risk) and presence (+ Risk) of predation risk averaged across levels of  $p\text{CO}_2$  concentration and temperature. Significant differences are indicated by an asterisk, see Table 4.3 for more detailed statistics.

Table 4.3: Three factor ANOVA on the effects of  $p\text{CO}_2$  (400 ppm and 1000 ppm), temperature (14 °C and 18 °C) and predation risk (- Risk, + Risk), on metabolic rate, energy intake and energy allocation of *L. littorea*. For factors, degrees of freedom (*df*), *F*-values and significance levels are provided, with *df* and mean square (MS) values provided for the residuals. Significant tests are shown in bold. \*\*\*  $p < 0.001$ ; \*\*  $0.001 < p < 0.01$ ; \*  $0.01 < p \leq 0.05$ . Results for Tukey HSD *post-hoc* tests are presented below the table.

Factor	Metabolic rate		Energy intake			Energy allocation		
	<i>df</i>	Oxygen uptake	<i>df</i>	Feeding	Assimilation	Intake	Somatic	Calcification
$p\text{CO}_2$ (OA)	1	0.66	1	<b>79.19 ***</b>	<b>39.72 ***</b>	<b>73.79 ***</b>	<b>111.24 ***</b>	<b>85 ***</b>
Temperature (T)	1	<b>12.84 ***</b>	1	0.45	0.69	0.16	0.04	2.24
Cue (C)	1	0.01	1	<b>54.38 ***</b>	1.06	<b>37.81 ***</b>	<b>30.3 ***</b>	<b>31.69 ***</b>
OA $\times$ T	1	1.65	1	<b>6.43 *</b>	4.01	1.88	<b>4.19 *</b>	<b>10.33 **</b>
OA $\times$ C	1	0.04	1	<b>28.1 ***</b>	1.57	<b>24.6 ***</b>	<b>4.31 *</b>	0.37
T $\times$ C	1	0.66	1	0.82	0.03	0.5	0.38	0.02
OA $\times$ T $\times$ C	1	0.03	1	<b>5.85 *</b>	0.06	<b>2.44</b>	0.5	0.06
Residuals (MS)	72	571	40	4.30	0.01	487.78	0.04	0.06
Tukey's <i>post-hoc</i> tests:								
$p\text{CO}_2$ (OA)	–			400 > 1000	400>1000	400 > 1000	400 > 1000	400 > 1000
Temperature (T)	18>14			–	–	–	–	–
Cue (C)	–			– Risk > + Risk	–	– Risk > + Risk	– Risk > + Risk	– Risk > + Risk
OA $\times$ T	–			400:14 = 400:18 > 1000:14 = 1000:18	–	–	400:14=400:18 > 1000:14 = 1000:18	400:18 > 400:14 > 1000:14 = 1000:18
OA $\times$ C	–			400:– Risk > all	–	400:– Risk > all; 400: + Risk > 1000: – Risk = 1000: + Risk	400:– Risk > 400: + Risk > 1000:– Risk = 1000: + Risk	–
OA $\times$ T $\times$ C	–			400:18:– Risk > 400:14:– Risk > all	–	–	–	–



Similar to *N. lapillus*, energetic intake for *L. littorea* largely reflected the patterns of feeding rate, however, some other differences were observed (described below) and hence for *L. littorea*, both feeding rates and energetic intake will be described separately.

Feeding rates demonstrated a significant three-way interaction between  $p\text{CO}_2$  concentrations, temperature and predation risk (Table 4.3; Figure 4.6a). In order to disentangle these results, the differences are first described in the presence and absence of predation risk, and then described together. In the absence of predation cues, we observed a significant interaction between  $p\text{CO}_2$  concentrations and temperature for feeding rates ( $F_{1,20} = 12.28$ ,  $p < 0.01$ ). This was due to a positive (significant) effect of temperature in control  $p\text{CO}_2$  concentrations, and a negative trend for increased temperature in elevated  $p\text{CO}_2$  conditions (Figure 4.6a). In the presence of predation risk, elevated  $p\text{CO}_2$  concentrations had a significant negative effect ( $F_{1,20} = 6.48$ ,  $p < 0.01$ ) on feeding rates, with temperature and their interaction showing no significant effect. Overall, this can be described as - any combination of the presence of predation risk, elevated  $p\text{CO}_2$  conditions and increased temperature - having a significant negative effect on *L. littorea* feeding rates (relative to control conditions, 400 ppm, 14 °C, - Risk), with the only positive effect exhibited by increased temperature under control  $p\text{CO}_2$  conditions in the absence of predation risk (Table 4.3; Figure 4.6a).

Energetic intake responses for *L. littorea* demonstrated a significant interaction between

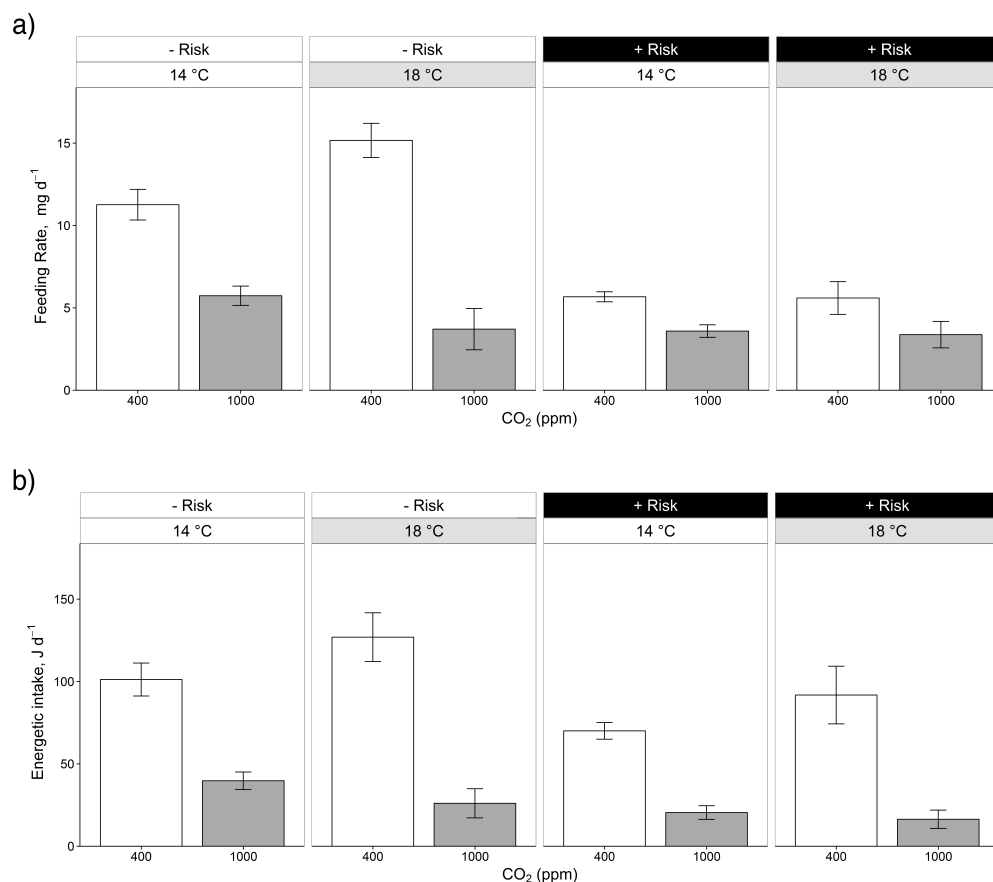


Figure 4.6: Mean ( $\pm$  S.E.) a) feeding rate ( $\text{mg d}^{-1}$ ), and b) energetic intake ( $\text{J d}^{-1}$ ) of *L. littorea*,  $n = 6$ . See Table 4.3 for significant differences.

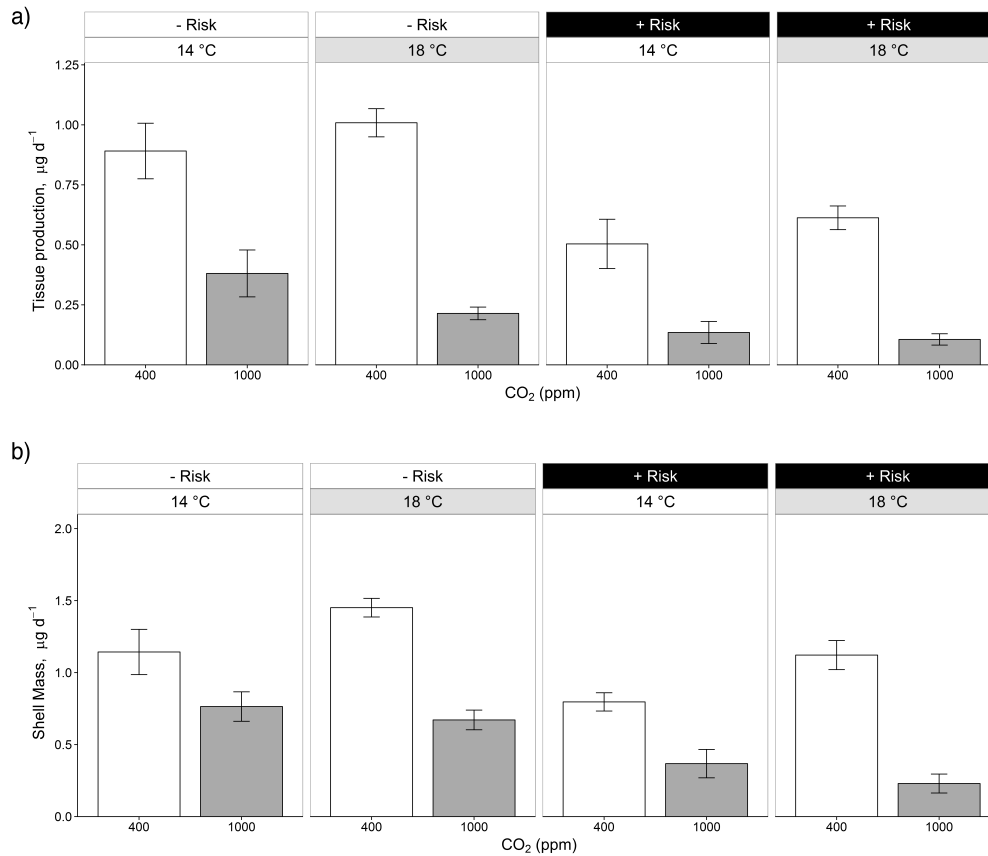


Figure 4.7: Mean ( $\pm$  S.E.) a) tissue production ( $\mu\text{g d}^{-1}$ ), b) change in length ( $\mu\text{m d}^{-1}$ ), and c) shell mass ( $\mu\text{g d}^{-1}$ ) of *L. littorea*,  $n = 6$ . See Table 4.3 for significant differences.

elevated  $p\text{CO}_2$  and predation cues (Table 4.3; Figure 4.6b). This was demonstrated by predation cues reducing energetic intake during control  $p\text{CO}_2$  conditions, but having no significant effect during elevated  $p\text{CO}_2$  conditions (Table 4.3; Figure 4.6b). Moreover, this was an antagonistic interaction (based on effect size), meaning predation risk had a reduced effect on energetic intake when in elevated  $p\text{CO}_2$  conditions. Temperature had no significant effect on energy intake (Table 4.3).

#### 4.3.6 *L. littorea* tissue production and shell mass change

Somatic growth in *L. littorea* reflected a similar pattern to that of feeding and energetic intake. We observed a significant interaction between  $p\text{CO}_2$  concentration and predation risk for tissue production (Table 4.3). Both elevated  $p\text{CO}_2$  conditions and predation cues had a significant negative effect on somatic growth relative to control conditions, however when at elevated  $p\text{CO}_2$  conditions, the presence or absence of predation risk had no significant effect on somatic growth (Table 4.3; Figure 4.7a). We also found a significant interaction between  $p\text{CO}_2$  concentration and temperature for tissue production (Table 4.3), due to the direction of the response to increased temperature going from a positive trend in ambient  $p\text{CO}_2$  conditions, to a negative trend in elevated  $p\text{CO}_2$  conditions (Figure 4.7a). Shell mass change for *L. littorea* demonstrated a significant interaction between  $p\text{CO}_2$  concentration and temperature, due to elevated  $p\text{CO}_2$  conditions reversing the positive response of temperature in a same manner as for tissue production (Table 4.3;

Figure 4.7b). Predation risk also had a significant negative effect on shell mass change (Table 4.3; Figure 4.7b).

#### 4.4 DISCUSSION

Our findings suggest important changes in the energetic allocation of intermediate consumers, *N. lapillus* and *L. littorea*, in response to ocean acidification and warming. This was demonstrated by decreases in their energy intake, through reduced feeding rates and assimilation efficiency, and increased energy expenditure due to higher maintenance costs. The risk of predation also suppressed feeding rates, however, the strength of this appeared to be inversely related to the physiological state of both *N. lapillus* and *L. littorea* respectively. Hence, as elevated  $p\text{CO}_2$  conditions and increased temperature reduced the prey state (in terms of reduced body tissue production), predation cues appeared to have an increasingly reduced effect on the intermediate consumers. Conversely, the predator in our food chain (*C. maenas*) demonstrated elevated feeding rates in the warm and acidic conditions, suggesting the possibility of enhanced top-down control in future oceans. Therefore, it is likely that our intermediate consumers will have to become less risk adverse in their foraging, potentially suffering increased predation, or risk starvation. Simultaneously, we anticipate these changes in the trophic dynamics will also have indirect positive influences on the abundance of basal resources through a combination of both trait-mediated indirect effects and density-mediated cascades. However, this may all be at the expense of the fitness of the intermediate consumers.

##### 4.4.1 Intermediate prey state

The increased energy demands and reduced energy intake of elevated  $p\text{CO}_2$  conditions and increased temperature resulted in limited somatic tissue production, and suggest that the intermediate consumers are facing a heightened risk of starvation with future climate change. As a consequence, our intermediate consumers will likely be forced to accept a greater risk of predation and undertake riskier behavioural choices in order to acquire a higher rate of energy intake. The strength of NCEs is highly dependent on the current state of the prey (Matassa & Trussell, 2014b). Prey with sufficient reserves can ‘wait out’ high risk periods, and then compensate with increased feeding during times (or places) of relative safety (Houston *et al.*, 1993), while prey with a high risk of starvation may be more willing to accept an increased risk of predation (McNamara & Houston, 1987; Heithaus *et al.*, 2007, 2008). It must be assumed that prey need to acquire some minimum amount of energy in order to survive (Lima & Bednekoff, 1999), presumably covering basic maintenance and overheads (Kooijman, 2010) and therefore, as environmental conditions drive energy budgets towards this minimum, we suggest that the strength of NCEs will be reduced.

The strength of NCEs will also be highly dependent on the environmental conditions under which the consumer currently resides, as well as their own physiological context (Matassa & Trussell, 2014a). It is possible that the positive effects of warming may offset the negative effects of predation risk when foraging can be achieved at a faster rate, reducing the time spent in high risk areas - ‘less vulnerability per joule’ (*sensu* Matassa & Trussell, 2014a). However, this assumes that the temperature increase keeps an organism within a favourable portion of their thermal performance curve (Stillman, 2003).

Should energy intake not scale with increasing energy demand (e.g. due to reduced prey quality, Harvey & Moore, *In Submission*), then any energy reserves will be depleted at a faster rate (Hochachka & Somero, 2002). We observed that any positive impacts associated with warming were generally negated or reversed when combined with elevated  $p\text{CO}_2$  concentrations (often with synergistic interactions). Elevated  $p\text{CO}_2$  concentrations not only reduced the feeding rate of the consumers directly (e.g. Appelhans *et al.*, 2012; Russell *et al.*, 2013; Harvey & Moore, *In Submission*), but also reduced their assimilation efficiency, likely due to a change in their gastric pH (e.g. Stumpp *et al.*, 2013). This is likely to result in an overall reduction in the energy available for performance activities in our intermediate consumers (e.g. protein synthesis, reproductive investment), with likely implications for both their fitness and trophic transfer of energy (Langenbuch & Pörtner, 2002; Trussell *et al.*, 2003; Stumpp *et al.*, 2011).

#### 4.4.2 *Induced-defences and fitness trade-offs*

The resilience of gastropods to crab predation is dependent on both their shell size and thickness (Palmer, 1985). Fast growth rates will enable them to achieve a greater size (but possess a thinner shell), and slow growth will allow for a thicker shell, but smaller shell length. Predator-induced defences represent an ecologically important phenotypically plastic trait that provides resistance to predation by an adaptive morphological shift (shell thickening) in response to temporal or spatial heterogeneity in predation risk (Tollrian & Harvell, 1998; Bourdeau, 2010). If, as our results suggest, a greater predation risk has to be accepted then the resilience of our consumers to predation becomes increasingly important. Induced defences are thought to be a passive by-product of their altered behaviour (i.e. their reduced activity and feeding) rather than an active physiological response to predation risk *per se* (Cowell & Crothers, 1970; Trussell *et al.*, 2003; Bourdeau, 2010). This means that induced defences are not necessarily energetically costly, since cheap lighter homogenous shell layers are deposited instead of expensive cross-lamellar layers (originating from a thickening of the aperture lip, Bourdeau, 2010). Subsequently, individuals experiencing predation risk should demonstrate similar responses to those with low food availability (in the absence of predation cues), i.e. by thickening their shell (not lengthening it), resulting in an overall increased shell strength (e.g. *Nucella lamellosa* Gmelin 1791, Bourdeau, 2010).

Whether predation risk or the environmental conditions are the reason for the reduced foraging that we observed, this will result in a slow growing gastropod that has a thicker shell (relative to its length), and an increased predation resistance in terms of shell strength. It is possible that elevated  $p\text{CO}_2$  conditions can reduce this resilience by either passive dissolution of the shell (when  $\Omega_{\text{calc}} < 1$ ) or by increasing the physiological costs at the site of calcification (Stumpp *et al.*, 2012a), resulting in a thinner shell relative to ambient  $p\text{CO}_2$  conditions. Hence, the induced defences of a gastropod will represent a net balance between passive thickening and the countervailing effects of ocean acidification. The mechanism of passive shell thickening will, however, also have fitness costs: developmentally, by limiting future somatic growth (Palmer, 1981), and (possibly) fecundity costs due to a reduced body size (e.g. body size correlates with clutch size in *Nucella lamellosa* Gmelin, 1791 and *N. emarginata* Deshayes, 1839, Spight & Emlen, 1976). Similarly, although a thicker shell may positively affect the survival of the individual, the slower growth rate and shell size mean that more time may have to be spent ‘at risk’ before a size refuge from predation can be reached (Thompson, 1975). Therefore, it is likely that shells may

still enable some resistance to predation, however, the population-level consequences of growth-limitation and reduced energy may have more important implications when considering the persistence of the intermediate consumers, including their role within the community.

#### 4.4.3 Community structure and trophic interactions

Despite the strength of NCEs being reduced in elevated  $p\text{CO}_2$  conditions and increased temperature, the resulting reduction in foraging is still likely to have positive trait-mediated indirect effects on the basal resources (i.e. similar to the presence of a predator), as well as density-mediated effects due to reduced consumer numbers should the consumers have to become less risk adverse. Generally, higher trophic positions are considered to be more sensitive to environmental change (Voigt *et al.*, 2003), with any changes having disproportionate effects on community composition across the trophic links (Schmitz *et al.*, 2003; Borer *et al.*, 2006). The predator in these food chains, *C. maenas*, represents a ubiquitous and voracious predator that can be considered as a (nearly) cosmopolitan species of the temperate regions of the world (Sprung, 2001; Trussell *et al.*, 2003; Griffen *et al.*, 2008), partially due to its broad physiological tolerances (Cohen *et al.*, 1995; Carlton & Cohen, 2003). These same physiological tolerances mean that *C. maenas* are expected to demonstrate a high tolerance to future climate change (particularly ocean acidification, Whiteley, 1999, 2011), and will be capable of at least maintaining or even increasing their top-down control of our intermediate consumers (e.g. Miller *et al.*, 2014). Therefore, this might not represent a novel (species-specific) mechanism, and it is possible that other predators demonstrating resilience to future climate change may also have the potential to maintain or increase their levels of top-down control.

Previous studies have also suggested that the basal resources used in this current study also show some tolerance to ocean acidification, with *F. serratus* expected to benefit from increased  $\text{CO}_2$  availability (Brodie *et al.*, 2014), and *M. edulis* capable of achieving physiological homeostasis given sufficient food availability (Thomsen *et al.*, 2012). Despite not observing any particularly prevalent responses to increased temperature in our basal resources, greater temperature increases are likely to have more of a negative influence on the basal resources than ocean acidification (Read & Cumming, 1967; Jones *et al.*, 2009; Jueterbock *et al.*, 2014), with aerial temperatures (in particular) likely to be a principal limiting factor to their performance (Schneider & Helmuth, 2007; Schneider, 2008), particularly at their southern range edges (Mieszkowska *et al.*, 2006; Jueterbock *et al.*, 2014). Therefore, with relatively resilient species at the top and bottom of our food chain, we suggest that it is the intermediate consumers that will be disproportionately impacted, with important implications for the stability of their populations (e.g. resource limitation stabilises populations, Tilman, 1996) and the ability of the middle of the food chain to absorb any additional perturbations (Murdoch & Oaten, 1975).

#### 4.4.4 Conclusion

Given the idiosyncratic responses that organisms demonstrate to future climate change conditions (Harvey *et al.*, 2013; Kroeker *et al.*, 2013b), this work promotes the need to move beyond single species responses and to simultaneously consider the inclusion of those species that are trophically linked. We suggest that future ocean acidification and

warming govern the diminishing energy budget of our intermediate consumers, which requires them to be less risk adverse, but ultimately more susceptible to predation. As a consequence, the strength of NCEs appear to reduce as energy reserves are exhausted, and the behavioural choices of the organisms become driven instead by the need to reduce starvation risk. Since most species reside in the middle of food chains (Trussell & Schmitz, 2012), it is likely that the effects of predation risk under future ocean acidification and warming will be inversely proportional to the ability of organisms to obtain energy and manage their energy budget. Therefore, as  $p\text{CO}_2$  concentrations and temperature increase, organisms on the edge of starvation may not respond to NCEs, however in fitness terms that may be the least of their worries. Hence, understanding the effects of ocean acidification and warming will require elucidating the relative importance of top-down and bottom-up effects, including the context-dependency of predation risk.

## EFFECTS OF OCEAN ACIDIFICATION, WARMING AND BIODIVERSITY ON THE FUNCTIONALITY OF MARINE ECOSYSTEMS

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### **Abstract**

There is a growing recognition that climate change research needs to increase the realism of experiments by moving beyond single species approaches towards assessing the effects of future climate change and altered biodiversity on the functioning of ecosystems. Primary production plays a central role in the structuring of marine food webs, and it is therefore important to understand how the effects of climate change will alter the rates and distribution of primary production, given that any changes to primary production could cascade through the ecosystem. Therefore, this study investigates the impacts of ocean acidification and warming on the responses of the microphytobenthos (MPB), a microalgal assemblage, and their key grazers in order to assess how several ecosystem processes (termed 'ecosystem multi-functionality') might be affected. It was found that the more species-rich grazing assemblages could promote resource utilisation relative to the less diverse monocultures. However, at elevated temperatures, maintaining the ecosystem functioning required a trade-off between ecosystem processes (primary production, MPB standing stock and resource utilisation), and importantly highlights that future climate change could alter the balance between these processes. Therefore, this study highlights the need to consider the multifunctionality of ecosystems in response to future climate change.





## 5.1 INTRODUCTION

There is now a general consensus that a positive relationship exists between biodiversity and ecosystem functioning, and that species loss could impact a range of ecosystem processes, including primary production and nutrient cycling (for reviews, see [Hooper \*et al.\*, 2005](#); [Balvanera \*et al.\*, 2006](#); [Cardinale \*et al.\*, 2006](#); [Hooper \*et al.\*, 2012](#); [Tilman \*et al.\*, 2014](#); [Gamfeldt \*et al.\*, 2015](#)). Biodiversity effects are typically attributed to one of two principal mechanisms: selection effects and complementarity ([Tilman \*et al.\*, 1997](#); [Loreau & Hector, 2001](#)). The ‘selection effect’ describes the dominance of a species with particular traits, that subsequently affects ecosystem processes, and the ‘complementarity effect’ describes the local deterministic processes which increase the performance of a community beyond what is expected from the performance of individual species raised alone ([Loreau & Hector, 2001](#)). Global environmental change, together with other human-induced perturbations ([Halpern \*et al.\*, 2008a](#)), means that there is now considerable interest in understanding how changes in species biodiversity and altered community structures will influence ecosystem functioning and the goods and services that natural systems provide to humans.

Ocean acidification and global warming represent two major drivers of environmental change that are predicted to fundamentally alter the structure and functioning of marine ecosystems ([Harley \*et al.\*, 2006](#); [Halpern \*et al.\*, 2008a](#)). Despite significant progress in understanding how ocean acidification and warming can affect the physiology and survival of individual species (for reviews, see [Harvey \*et al.\*, 2013](#); [Kroeker \*et al.\*, 2013b](#)), the effects on community dynamics and ecosystem function remains poorly understood ([Alsterberg \*et al.\*, 2013](#); [Kroeker \*et al.\*, 2013a](#); [Eklöf \*et al.\*, 2015](#)). Consequently, there is a growing recognition that one avenue of climate change research is to increase the realism<sup>1</sup> of experiments by moving beyond single species approaches towards an understanding of the effects of future climate change on communities and the functioning of ecosystems ([Harley \*et al.\*, 2006](#); [Walther, 2010](#); [Harvey \*et al.\*, 2014](#); [Eklöf \*et al.\*, 2015](#)). Primary production plays a central role in structuring marine food webs ([Hunt Jr & McKinnell, 2006](#); [Shurin \*et al.\*, 2006](#)), and it is therefore important to understand how the effects of climate change will alter the rates and distribution of primary production ([Brown \*et al.\*, 2010](#)), given that any changes to primary production could cascade through the ecosystem (e.g. [Beaugrand \*et al.\*, 2003](#)).

In this chapter, the microphytobenthos (MPB) and key grazer responses to ocean acidification and warming are investigated. MPBs are a microalgal assemblage that is considered to be one of the main primary producers in many intertidal and shallow sub-tidal environments ([Tang & Kristensen, 2007](#)), as well as the base of many food-webs, including being a particularly important food resource for grazers ([Thompson \*et al.\*, 2000, 2004](#)). On the rocky shore, MPBs will form an extracellular biofilm matrix on any submerged hard substrate (i.e. epilithic biofilm), and are typically comprised of benthic microscopic algae including cyanobacteria, diatoms, benthic dinoflagellates, and microscopic life-history stages of macroalgae ([Brodie \*et al.\*, 2014](#)). It is possible that MPB communities

<sup>1</sup> Context and definition for ‘realism’. Here realism is used *sensu* [Dupont & Pörtner, 2013](#) which stated that “experiments must become more sophisticated and realistic. No single experiment can capture the complexity, so a variety of approaches will be needed.” As such, realism is used here to represent the need for unravelling ecological interactions and community-level responses to climate change, and represents one avenue of investigation. It does not, however, imply that single-species investigations and mechanistic underpinnings will no longer be valuable to our understanding.

could be a 'winner' under future climate change scenarios, with increased temperature (up to an optimum) having positive effects on photosynthesis (Blanchard *et al.*, 1996; Vieira *et al.*, 2013), and the increased availability of CO<sub>2</sub> enhancing biofilm production (Johnson *et al.*, 2011; Lidbury *et al.*, 2012). However, species-specific mechanisms, such as the presence of a carbon concentrating mechanism, and different sensitivities to temperature and ocean acidification will partially dictate individual species responses (Raven *et al.*, 2011; Koch *et al.*, 2013). Regardless, any positive response by the MPB has the potential to increase their carbon export (increased autochthonous productivity) and have a considerable influence on the net carbon status of the ecosystem (Brodie *et al.*, 2014).

Climate change has been demonstrated to alter species responses through changes in the relative importance of top-down and bottom-up effects (Hoekman, 2010; Kratina *et al.*, 2012; Miller *et al.*, 2014), and subsequently a more realistic view into the responses of MPBs needs to consider other components of the community. Herbivores are anticipated to play a strong role in the recruitment and initial growth of primary producers (Hawkins & Hartnoll, 1983), especially following a disturbance (Dethier, 1984). Subsequently, herbivores have the potential to influence the diversity, community composition, biomass and productivity of primary producers. In addition, ocean warming (for example) is expected to strengthen consumer-driven control, since heterotrophic metabolism is considered to be more sensitive to increases in temperature when compared to autotrophic metabolism and production (Allen *et al.*, 2005; Lopez-Urrutia *et al.*, 2006; O'Connor *et al.*, 2009). This increased top-down control could mediate any positive responses by the MPBs to ocean warming and acidification (e.g. Alsterberg *et al.*, 2013), resulting in a reduced standing stock of MPBs biomass, and potentially shift the carbon balance of the ecosystem towards greater heterotrophy (Hicks *et al.*, 2011).

Such changes in the carbon balance would mean that the ecosystem functioning of the MPB in terms of biomass and primary production would be negatively affected, however, the same top-down control could also promote photo-autotrophic aspects of the biofilm by grazers removing unproductive heterotrophic biofilm canopy (Skov *et al.*, 2010). Moreover, the changes in MPB could actually enhance diversity effects for the grazers by limiting resources (forcing niche partitioning; Finke & Snyder, 2008), or facilitate niche partitioning by stimulating the availability of different resources (e.g. fertilisation by nutrient release or excretion, Sherr *et al.*, 1986), and thus promote secondary production (as an alternative ecosystem process). Such trade-offs between ecosystem processes mean that the effects of biodiversity cannot be based on single functions and instead need to be considered in terms of ecosystem 'multi-functionality' (Byrnes *et al.*, 2014). As such, it is expected that the consumer resource dynamics of grazers and MPBs communities will display a complex interplay of direct and indirect effects.

With recent research showing that the community-level effects of reduced biodiversity could actually equal or outweigh the effects of anthropogenically-induced environmental change (Hooper *et al.*, 2012; Tilman *et al.*, 2012; Eklöf *et al.*, 2015), there is now a need to understand the relative importance of biodiversity and climate change (including any interactions between them), in order to determine the responses of communities and their ecosystem functioning (Eklöf *et al.*, 2015). In this study, we manipulated the richness and composition of three epilithic grazers (*Gibbula umbilicalis*, *Littorina littorea* and *Patella vulgata*) and quantified the interactive effects of elevated pCO<sub>2</sub> concentrations, increased temperature, and consumer (grazer) identity and richness on the biomass and

Table 5.1: Abundance based on the metabolic biomass of the three consumers (*Gibbula umbilicalis*, *Littorina littorea*, *Patella vulgata*), in order to account for differences in body size.

	<b>Monoculture</b>			<b>Polyculture</b>		
	<b><i>Gibbula</i></b>	<b><i>Littorina</i></b>	<b><i>Patella</i></b>	<b><i>Gibbula</i></b>	<b><i>Littorina</i></b>	<b><i>Patella</i></b>
<b>Abundance</b>	12	15	3	4	5	1

ecosystem functioning of the MPBs. Specifically, we used a mesocosm-based system to quantify the treatment effects on the structure (species composition) and functioning (biomass, productivity and secondary production) of the MPB communities. We first hypothesised that the community structure and functioning will be predominantly dictated by grazer richness, rather than by grazer identity. Secondly, that the increased ‘cost of living’ associated with elevated  $p\text{CO}_2$  and temperature conditions will cause a shift towards heterotrophy and will reduce the structure and functioning of the MPB communities, regardless of the grazer composition or richness.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Experimental design

The experiment consisted of three crossed treatments, ocean acidification (two levels, ‘ambient’, 400 ppm  $p\text{CO}_2$  and ‘acidified’, 1300 ppm  $p\text{CO}_2$ ), ocean warming (two levels, ‘ambient’ 14 °C and ‘warmed’, 18 °C), and species composition (five levels, described below). The experiment ran for eight weeks,  $n = 6$ , making a total of 120 tanks. Levels of temperature and  $p\text{CO}_2$  were chosen based on the IPCC [2013] RCP 8.5 (worst-case) scenario for the year 2100. Species composition involved the manipulation of the richness and identity of three molluscan grazers (the topshell *Gibbula umbilicalis*, the periwinkle *Littorina littorea* and the limpet *Patella vulgata*). The five levels of the species composition treatments involved a no grazer control, three single species treatments (monocultures of *G. umbilicalis*, *L. littorea* and *P. vulgata*) and a mixture of all three species (polyculture). The three consumer species used here are all abundant, ubiquitous and coexisting inhabitants of rocky shores around much of the north-east Atlantic. A substitutive design was employed with consumer numbers standardised across treatments to account for differences in body using allometric scaling of metabolic rate (B) to body mass (M) with  $B = M^{3/4}$  (West *et al.*, 1997) termed metabolic biomass. We determined metabolic biomass by sacrificing individuals collected at the same time as our experimental individuals (animal collection outlined in section 5.2.2). The standardised metabolic biomass meant that every individual of *P. vulgata* was equivalent to four individuals of *G. umbilicalis* and five individuals of *L. littorea*. Similarly, to maintain metabolic biomass, monocultures of species had three times the individuals of the polyculture (see Table 5.1).

The experiment was carried out using six semi-recirculating aquarium systems, each system comprised of 10 experimental tanks (35 L, 49 × 39 × 26 cm, Figure 5.1a) independently supplied from a header tank, making 60 experimental tanks in total. Due to logistical reasons of space, the experiment had to be carried out in two temporal blocks (each eight weeks long), with three replicates of the fully factorial design in each temporal block. Three aquarium systems were maintained at ‘ambient’ (14 °C) temperatures and

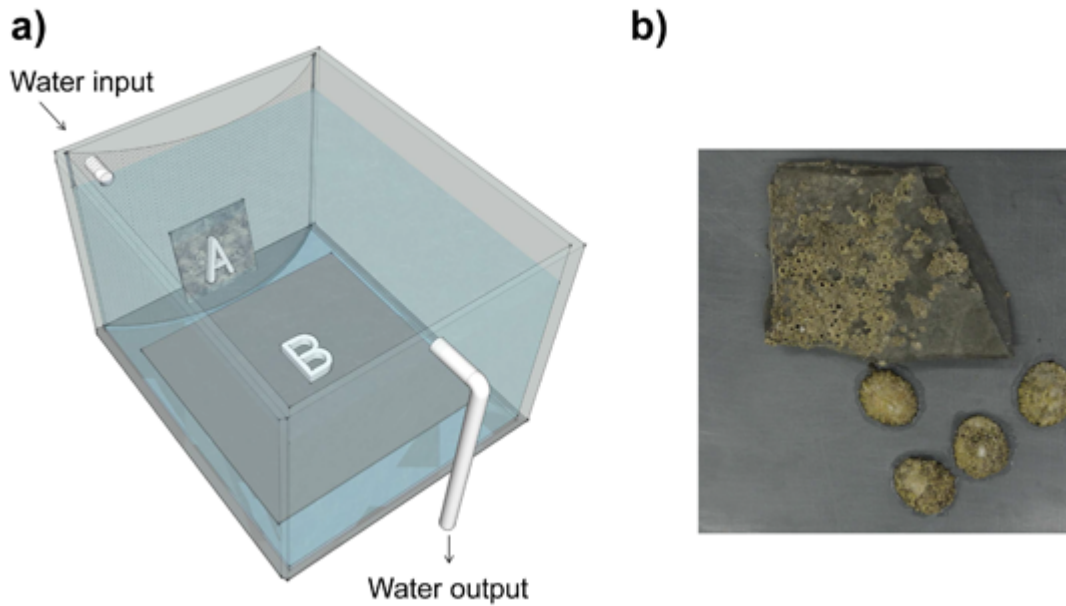


Figure 5.1: Schematic diagram of each experimental tank, specifically highlighting: Tile 'A' - a seed source for MPB community, and Tile 'B' - the tile on which responses were tested. See text for full description. b) Example of one of the tiles used as Tile 'B' exhibiting spatial heterogeneity with empty *Patella vulgata* shells and some slate rock covered in *Chthamalus montagui* tests, as well as flat surface.

the remaining three at 'warmed' (18 °C) temperatures (Teco SeaChill TR-20 Aquarium Chiller with additional heater kit, Ravenna, Italy). Experimental tanks were then randomly assigned to either be 'acidified' or 'ambient'  $p\text{CO}_2$  conditions (five of each per aquarium system), and the pH of each tank was set by continuous bubbling of a known air- $\text{CO}_2$  mix (LiCOR IRGA LI-820, Lincoln, NE USA). Both the header and footer tanks were suitably aerated in order to ensure that the seawater was de-gassed before being re-circulated. Finally, the five levels of species composition (no grazer, three monocultures and the poly-culture) were randomly assigned between treatments.

Each experimental tank contained two tiles, tile 'A' (150 × 150 mm) possessed a natural MPB community used to provide a seed for MPB in the tank, and was placed behind a perforated barrier to prevent the consumers from feeding on it (see Figure 5.1a). The natural MPB communities on tile 'A' were acquired by leaving them in the low intertidal for five weeks and placed into the experimental tanks one week prior to the start of the experiment. These tiles were used for no further part of this experiment, and subsequently will not be discussed further. Tile 'B' (240 × 240 mm) was a blank tile (in terms of MPB) and hereafter, any responses associated with MPB are referring to this tile (see Figure 5.1a and 5.1b). Tile 'B' was placed into the experimental tank one week before the start of the experiment, allowing a MPB community to establish on the blank panels under the relevant experimental treatment. Tile 'B' also contained spatial heterogeneity (four empty *P. vulgata* shells, shell length 35-45 mm, a piece of slate rock, 77-96 cm<sup>2</sup> covered in empty adult *Chthamalus montagui* tests, and the remaining homogeneous surface of the tile, see Figure 5.1b) as this has been demonstrated to promote differential resource utilisation patterns (i.e. niche partitioning, Griffin *et al.*, 2009). Hereafter, these substrate types will be referred to as 'Hummocks', 'Barnacles' and 'Tile' respectively.

### 5.2.2 Animal collection

The experimental molluscs, adult *G. umbilicalis* (shell length  $12.56 \pm 0.07$  mm [S.E.]), adult *L. littorea* (shell length  $11.26 \pm 0.06$  mm [S.E.]) and adult *P. vulgata* (shell length  $39.76 \pm 0.37$  mm [S.E.]) were collected from Aberystwyth, Wales, UK ( $52^{\circ} 24' 54.9''$  N,  $4^{\circ} 05' 28.7''$  W) during July and September 2014 (for their respective temporal block). Following collection, all three species were maintained (separately) in a flowing aquarium system for one week before being transferred into the experimental system.

### 5.2.3 Carbonate chemistry

Carbonate chemistry parameters were monitored by measuring  $\text{pH}_{\text{NBS}}$  (Mettler-Toledo SevenGo Pro pH meter with Inlab 413SG probe, Mettler-Toledo, Germany), temperature and salinity (WTW Cond 3210, WTW, Germany) daily, and by measuring total alkalinity ( $A_T$ , Hanna HI 755 Alkalinity Checker, Leighton Buzzard, UK) weekly. In order to calculate the additional carbonate chemistry parameters, following Nisumaa *et al.* [2010], the dissolved inorganic carbon ( $C_T$ ) was calculated using the software CO2SYS (Lewis & Wallace, 1998), with the measured  $\text{pH}_{\text{NBS}}$  and  $A_T$  as the input variables. Subsequently, the additional carbonate system parameters were calculated using the R package seacarb (Lavigne & Gattuso, 2010) using the calculated  $C_T$  and measured  $A_T$  (Table 5.1). For both CO2SYS and seacarb, we used disassociation constants from Mehrbach *et al.* [1973], as adjusted by Dickson & Millero [1987], and  $\text{KSO}_4$  using Dickson [1990] (Table 5.2). Light conditions were adjusted weekly to maintain natural light:dark cycles with aquarium lighting (Plant Pro T5 54W, Arcadia, Redhill, UK), seawater was replenished weekly, and fresh water added (every two to three days) to account for any salinity increases due to evaporation.

### 5.2.4 Response of MPB community to ocean warming and acidification

#### Photophysiology

Photophysiology of the MPB communities was quantified by chlorophyll (Chl) fluorescence measurements using rapid light curves (RLCs, White & Critchley, 1999) with a Diving PAM fluorometer (Walz, Effeltrich, Germany). These measurements were carried out using the 'no grazer control' tiles, and each tile had nine measurements taken, with the position on the tile designated by random number table. However, the positions were stratified so that three measurements (each) were taken across the spatial heterogeneity of the tile (i.e. on the Hummock, Barnacles and Tile). The RLCs were constructed based on 12 actinic increasing light levels (6, 23, 62, 110, 175, 255, 339, 474 and 621  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), each of 10 s duration, with no prior dark adaptation (as suggested by Perkins *et al.*, 2010b). This approach provides a methodology of relating the rate of photosynthetic electron transport (ETR) to photon flux density (PFD). Three key parameters are produced by this method: (1)  $\alpha\text{ETR}$  (electrons/photons), the initial slope of the RLC that is related to quantum efficiency of photosynthesis; (2)  $\text{ETR}_m$  ( $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ ), the maximum electron transport rate; and (3)  $E_k$  ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), the minimum saturating irradiance. These three measures are estimated using the model of Platt *et al.* [1980], whereby  $\alpha$  is calculated from fitting Equation 5.1, which also provides



Table 5.2: Mean ( $\pm$  SE) seawater properties.  $\text{pH}_T$ , temperature, salinity, and total alkalinity ( $A_T$ ) are measured values. Seawater  $\text{pCO}_2$ , dissolved inorganic carbon ( $C_T$ ), bicarbonate ( $\text{HCO}_3^-$ ), carbonate ( $\text{CO}_3^{2-}$ ), carbon dioxide ( $\text{CO}_2$ ), saturation states for calcite ( $\Omega_{\text{calcite}}$ ) and aragonite ( $\Omega_{\text{aragonite}}$ ) are values calculated using the carbonate chemistry system analysis program CO2SYS and the R package seacarb.

Target:	pH <sub>T</sub>	Temp (°C)	Salinity (psu)	A <sub>T</sub> (μmol kg <sup>-1</sup> )	pCO <sub>2</sub> (μatm)	C <sub>T</sub> (μmol kg <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (μmol kg <sup>-1</sup> )	CO <sub>3</sub> <sup>2-</sup> (μmol kg <sup>-1</sup> )	CO <sub>2</sub> (μmol kg <sup>-1</sup> )	Ω <sub>calcite</sub>	Ω <sub>aragonite</sub>
14 °C 400 ppm	Block 1	8.05 ± 0.01	33.47 ± 0.13	2296.76 ± 11.37	574.11 ± 13.08	2131.52 ± 10.55	1982.11 ± 9.68	127.21 ± 1.59	22.2 ± 0.52	3.05 ± 0.04	1.95 ± 0.02
	Block 2	8.08 ± 0.01	33.3 ± 0.15	2328 ± 17.86	529.58 ± 9.94	2150.04 ± 14.32	1993.2 ± 12.1	136.33 ± 3.44	20.51 ± 0.39	3.27 ± 0.08	2.09 ± 0.05
	Overall	8.07 ± 0.01	33.39 ± 0.57	2312.38 ± 14.99	551.84 ± 12.14	2140.78 ± 12.48	1987.65 ± 10.81	131.77 ± 2.76	21.36 ± 0.48	3.16 ± 0.07	2.02 ± 0.04
14 °C 1300 ppm	Block 1	7.71 ± 0.01	33.6 ± 0.13	2296.76 ± 11.37	1354.59 ± 35.7	2256.73 ± 13.51	2142.53 ± 13.07	62.13 ± 1.23	52.07 ± 1.37	1.49 ± 0.03	0.95 ± 0.02
	Block 2	7.72 ± 0.01	33.51 ± 0.15	2328 ± 17.86	1314.05 ± 27.76	2284.78 ± 16.6	2169.63 ± 15.6	64.49 ± 1.62	50.66 ± 1.06	1.55 ± 0.04	0.99 ± 0.02
	Overall	7.72 ± 0.01	33.56 ± 0.57	2312.38 ± 14.99	1334.32 ± 31.65	2270.75 ± 15.1	2156.08 ± 14.36	63.31 ± 1.43	51.36 ± 1.21	1.52 ± 0.03	0.97 ± 0.02
18 °C 400 ppm	Block 1	8.12 ± 0.01	33.21 ± 0.14	2390.28 ± 4.54	500.95 ± 9.77	2170.57 ± 5.73	1987.14 ± 7.03	166.17 ± 2.4	17.26 ± 0.35	4 ± 0.06	2.58 ± 0.04
	Block 2	8.14 ± 0.01	34.3 ± 0.17	2449.33 ± 29.04	507.4 ± 14.53	2214.19 ± 21.42	2019.1 ± 16.38	177.5 ± 6.22	17.58 ± 0.53	4.27 ± 0.15	2.75 ± 0.1
	Overall	8.13 ± 0.01	33.76 ± 0.56	2419.81 ± 21.15	504.18 ± 12.18	2192.38 ± 15.93	2003.12 ± 12.74	171.84 ± 4.75	17.42 ± 0.44	4.13 ± 0.11	2.67 ± 0.07
18 °C 1300 ppm	Block 1	7.75 ± 0.01	33.25 ± 0.14	2390.28 ± 4.54	1313.47 ± 24.78	2324.06 ± 6.41	2200.1 ± 6.71	78.76 ± 1.37	45.2 ± 0.88	1.89 ± 0.03	1.22 ± 0.02
	Block 2	7.77 ± 0.01	33.54 ± 0.17	2449.33 ± 29.04	1276.23 ± 33.31	2373.68 ± 25.48	2245.13 ± 23.46	84.33 ± 3.13	44.22 ± 1.27	2.03 ± 0.08	1.31 ± 0.05
	Overall	7.76 ± 0.01	33.4 ± 0.56	2419.81 ± 21.15	1294.85 ± 29.05	2348.87 ± 18.83	2222.62 ± 17.46	81.55 ± 2.43	44.71 ± 1.08	1.96 ± 0.06	1.27 ± 0.04

estimates for  $ETR_{mPot}$ , the maximum potential light-saturated electron transport rate, and  $\beta$ , the photoinhibition parameter (Platt *et al.*, 1980).

$$ETR = ETR_{mPot} \times \left(1 - e^{-\frac{\alpha - PFD}{ETR_{mPot}}}\right) \times e^{-\frac{\beta - PFD}{ETR_{mPot}}} \quad (5.1)$$

$ETR_m$  is then calculated from Equation 5.2, and  $E_k$  is calculated from Equation 5.3.

$$ETR_m = ETR_{mPot} \times \left(\frac{\alpha}{\alpha + \beta}\right) \times \left(\frac{\beta}{\alpha + \beta}\right)^{\frac{\beta}{\alpha}} \quad (5.2)$$

$$E_k = \frac{ETR_m}{\alpha} \quad (5.3)$$

## Growth

Total Growth and MPB resource type ( $\mu\text{g Chl a cm}^{-2}$ ) was measured using a Benthos-Torch fluorometer (bbe Moldaenke GmbH, Schwentinental, Germany) which utilises a specific excitation spectra of Chl fluorescence for particular photosynthetic antenna pigments, and thus allows the characterisation of MPB into ‘resource types’ of green (Chlorophyta), blue-green (Cyanobacteria) and diatoms (e.g. Beutler *et al.*, 2002). These measures were also carried out on the ‘no grazer control’ tiles with nine replicates per tile with random stratified sampling across the three surface types (i.e. Hummock, Barnacles, and Tile) in the same manner as for the PAM fluorometry. Although the tiles were allowed one week to establish a MPB community, we outline growth as relative to a blank tile (i.e. zero) and thus simply taken as the MPB present at the end of the experiment. Finally, MPB growth was standardised by surface area of the tile using surface area for the tile, and the tinfoil method (see Marsh Jr, 1970) for the Hummocks and Barnacles, and growth was reported as  $\text{mg Chl a d}^{-1}$ .

## Primary productivity

The MPB community primary production was measured by submerging each tile in a tank of seawater controlled in their respective temperature and  $p\text{CO}_2$  treatments, taking dissolved oxygen measurements (Orion Star A223 DO with polarographic  $\text{O}_2$  electrode, Thermo Scientific, Waltham, MA USA), following the methodology of Noël *et al.* [2010]. Oxygen measurements were taken three times. The first measurement was taken to assess initial dissolved oxygen concentration. Immediately afterwards, each tank was covered with opaque plastic sheets to create an artificial darkness. After one hour the sheets were removed and a second measurement was taken. The tanks were then left exposed for another 1-hour period under natural daylight aquarium lighting (Aquabeam 600 Ultima, Aquaray, TMC, London, UK) after which a third measurement was taken. During this time, the tanks were mechanically stirred (while sealed) to prevent stratification. The difference in dissolved oxygen concentration between the measurements taken respectively at the beginning and end of the dark period were used to calculate the community respiration.

The measurements taken respectively for the light period were used to calculate the net primary productivity. Measurements were then multiplied by the solubility coefficient for oxygen (corrected for temperature and salinity, Harvey, 1955) and the respirometer volume, for standard temperature and (dry) pressure (STPD). Gross primary productivity (GPP) was calculated as the sum of net primary productivity and community respiration, with results expressed as  $\mu\text{mol O}_2 \text{ h}^{-1}$  STPD.

### 5.2.5 Response of grazing assemblage to ocean warming and acidification

#### Consumer metabolism

Before the start of the experiment, each experimental *G. umbilicalis*, *L. littorea* and *P. vulgata* was labelled with a numbered, coloured queen bee tag (EH Thorne Ltd, Rand, UK) for identification. Oxygen uptake, as a proxy for metabolic rate, was measured using the same methodological approach as in Chapter 3 and Chapter 4. Individuals were placed in blacked out 250 ml respirometers located in water baths that maintained respective temperature and  $p\text{CO}_2$  treatments. The respirometers were left open (but covered) for one hour to remove handling stress, and rates of oxygen uptake were taken (Orion Star A223 DO with polarographic  $\text{O}_2$  electrode, Thermo Scientific, Waltham, MA USA) as the difference in dissolved oxygen between the starting measurement (immediately before sealing the respirometers) and the final measurement (taken after the respirometers were sealed for 1 hour). Final oxygen uptake was expressed in terms of  $\mu\text{mol O}_2 \text{ h}^{-1}$  STPD. Individuals from the polyculture were measured in the same manner as the monoculture, with the oxygen uptake measured for each individual separately.

#### Community respiration

Community respiration rates of the consumers in each tank were taken as the sum of the oxygen uptake rates of each replicate experimental tank, and multiplied by the number of consumers, as this standardised them by metabolic biomass (see Section 5.2.1; Table 5.1). This community respiration rate was measured in  $\mu\text{mol O}_2 \text{ h}^{-1}$  STPD, and was used to be indicative of the overall energy demand of the consumers within the tank (or community).

#### Consumer net effect rates

Consumer net effects were measured as the final standing MPB biomass of each monoculture and polyculture tile, subtracted from the no grazer control tiles, within each of the four crossed  $p\text{CO}_2$  and temperature treatments. MPB biomass was quantified in the same manner as for the no grazer control tiles using the BenthosTorch fluorometer (see section 5.2.4). Despite the principal influence of the consumers on the MPB being consumption, the effect of the consumers was described as the net effect because we were unable to disentangle gross MPB consumption from positive effects, such as MPB stimulation by the release of nutrients, excretion of labile organic compounds (Sherr *et al.*, 1986) or trapping of microalgal food particles in the pedal mucus of the gastropods (Davies *et al.*, 1992).



### 5.2.6 Testing diversity effects

Transgressive overyielding occurs when the ecosystem process in a polyculture actually exceeds the best performing species of the monocultures, and was tested for by comparing the polyculture with the best-performing monoculture (three-way ANOVA,  $p\text{CO}_2 \times \text{Temperature} \times \text{Grazing assemblage}$ , followed by Tukey HSD *post-hoc* test). Essentially, transgressive overyielding provides an indication as to whether species richness effects surpass that of species identity (Griffin *et al.*, 2009). Non-transgressive overyielding describes when the magnitude of a particular ecosystem process in a multi-species mixture (i.e. the polyculture) exceeds that of the mean value of the component species individually (monocultures). We explicitly tested for this using a student's *T*-test between the polyculture and the additive null model.

### 5.2.7 Ecosystem processes of the MPB

Gross primary productivity (as described above) and the MPB standing stock (the remaining MPB biomass following the grazing treatments, i.e. inverse of the net effects) were measured for all of the consumer treatments in order to test how elevated  $p\text{CO}_2$ , increased temperature, species identity and richness affected the ecosystem functioning of the MPB communities. Non-transgressive and transgressive overyielding (above) was tested in all cases.

### 5.2.8 Statistical analysis

Statistical analyses were conducted using R (R Development Core Team, 2012). In all cases data were normally distributed (Kolmogorov-Smirnov) as well as displaying homogeneity of variance (Levene) ( $p > 0.05$ ). Two-way factorial ANOVA - with an additional random effect for the temporal block - were used to test for possible differences among all measurements between treatments. Tukey HSD *post-hoc* tests were used to test pairwise differences. As previously stated, three-way ANOVA was used for testing Transgressive overyielding, and student's *T*-test for testing for non-transgressive overyielding (section 5.2.6).

## 5.3 RESULTS

### 5.3.1 Direct responses of the MPB community to ocean warming and acidification

#### Photophysiology

The photophysiology of the MPB was altered by the environmental conditions, this included the RLCs as a whole (Figure 5.2), dictated by several of the key parameters of the RLCs.  $E_k$ , the light saturation coefficient, was significantly increased by elevated  $p\text{CO}_2$  conditions (Table 5.3; Figure 5.3a), but was not significantly affected by either increased temperature (Table 5.3; Figure 5.3b) or their interaction (Table 5.3). The maximum rate of electron transport ( $\text{ETR}_m$ ) demonstrated a significant block effect, with the second temporal block showing an overall increase by  $\sim 2.9 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$  (relative to the first temporal block), however, since the pattern was similar across treatments, the results

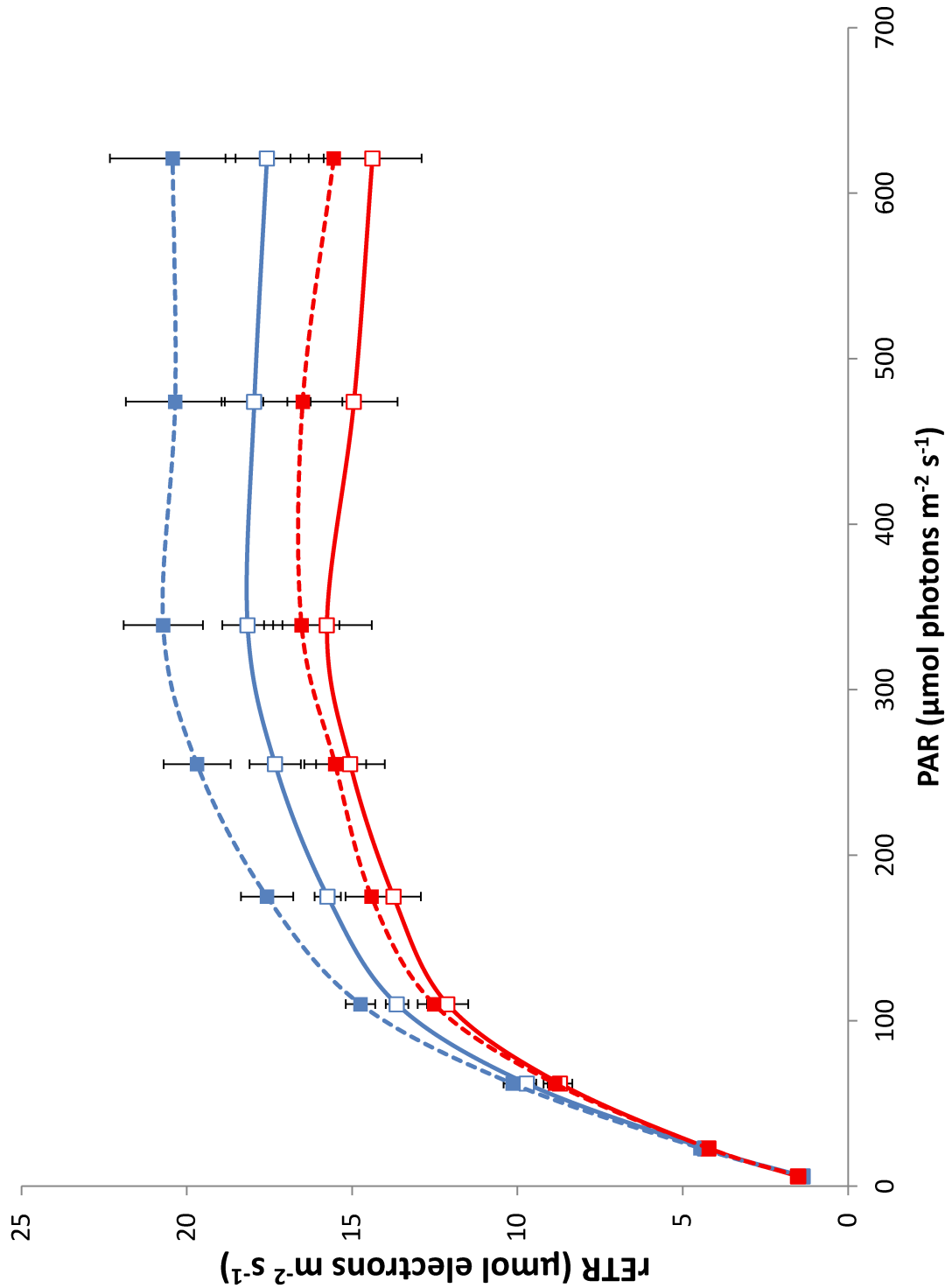


Figure 5.2: RLCs of the 'no grazer control' tiles in response to different levels of pCO<sub>2</sub> and temperature. Ambient treatments (14 °C and 400 ppm, blue solid line), increased temperature (18 °C and 400 ppm, red solid line), elevated pCO<sub>2</sub> conditions (14 °C and 1300 ppm, blue dashed line) and the combination of elevated pCO<sub>2</sub> conditions and increased temperature (18 °C and 1300 ppm, red dashed line).

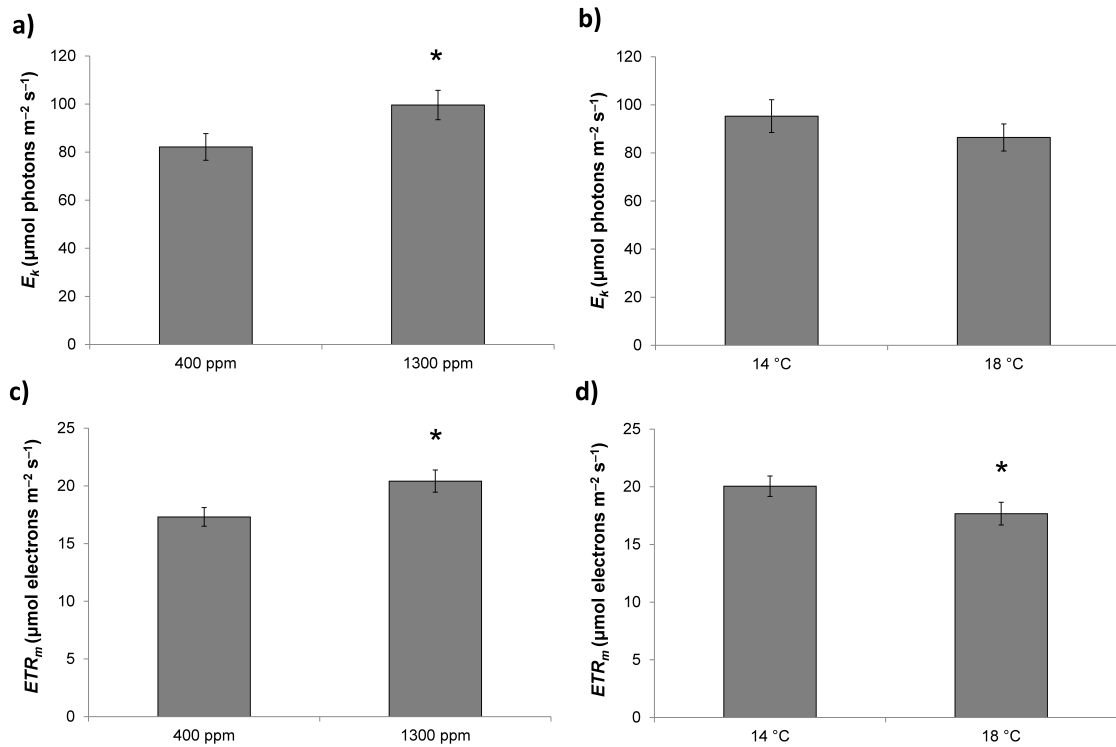


Figure 5.3: Mean ( $\pm$  S.E.) (a-b)  $E_k$  (the light saturation coefficient) and (c-d)  $ETR_m$  (maximum electron transport rate) of the MPB in response to (a and c) different levels of  $pCO_2$  concentration (400 ppm and 1300 ppm) and (b and d) different levels of temperature (14 °C and 18 °C). Significant differences are indicated by an asterisk.

were pooled. Elevated  $pCO_2$  conditions significantly increased  $ETR_m$ , while increased temperature significantly reduced  $ETR_m$  (Table 5.3; Figure 5.3c and d). The parameter  $\alpha ETR$ , the light-limited photosynthetic efficiency, was not significantly affected by elevated  $pCO_2$  conditions, increased temperature or their interaction (Table 5.3).

### MPB growth

Total MPB growth or settlement (but hereafter described as growth) was significantly augmented by increased temperature, but was not affected by either elevated  $pCO_2$  conditions or their interaction (Table 5.3; Figure 5.4a).

### MPB resource types

The MPB was mostly comprised of diatoms, and so diatoms followed the same pattern as the overall MPB, with temperature increasing growth (Table 5.3; Figure 5.4b). The growth of both the green and cyanobacteria MPB was not significantly affected by elevated  $pCO_2$  conditions, increased temperature, or their interaction (Table 5.3; Figure 5.4c and d).

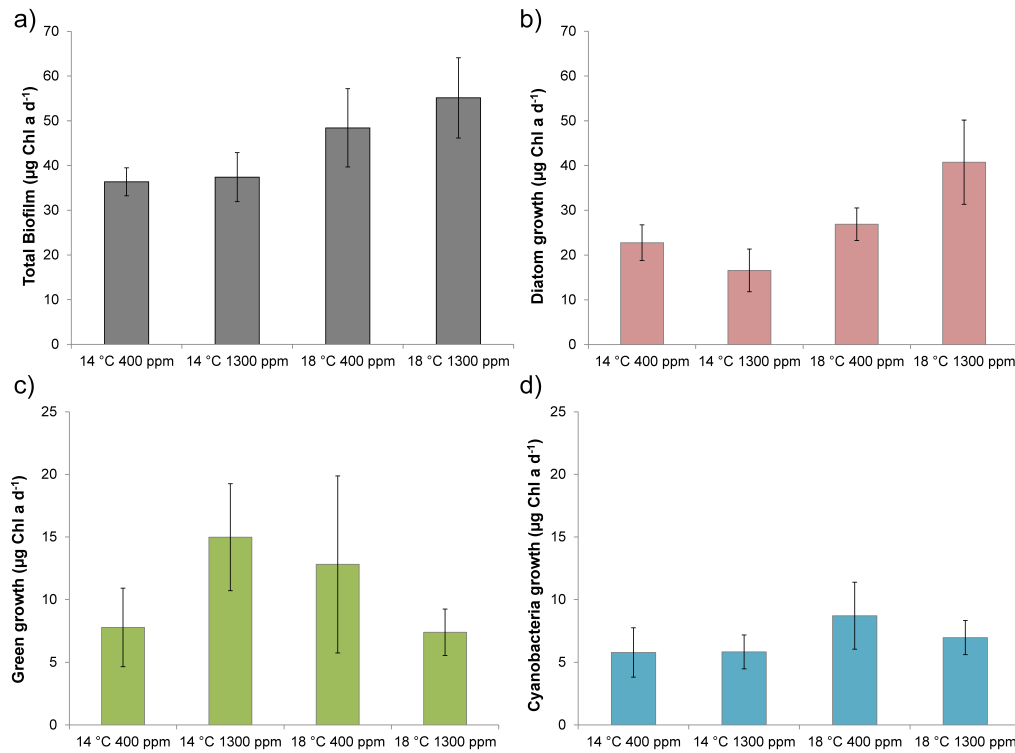


Figure 5.4: Mean ( $\pm$  S.E.) MPB growth ( $\mu\text{g Chl a d}^{-1}$ ) for a) total MPB, b) diatoms, c) green, and d) cyanobacteria,  $n = 6$ . Note different y-axis for panel a-b compared to c-d. See Table 5.3 for significant differences.

### Does MPB growth differ between substrate types?

The total MPB growth was not significantly affected by the substrate type (i.e. Hummocks, Barnacles, and Tile) on which the MPB settled ( $F_{2,66} = 1.72$ ,  $p = 0.19$ , Figure 5.5a). In terms of specific resource type, diatom growth was not significantly affected by substrate type ( $F_{2,66} = 0.81$ ,  $p = 0.45$ , Figure 5.5b), but the growth of both the cyanobacteria and green MPB were significantly altered by the substrate type which they settled on. This 'substrate choice', however, was not influenced by either increased temperature and/or  $p\text{CO}_2$  ( $p > 0.50$  in all cases). Cyanobacteria growth was significantly greater on the more spatially heterogeneous Hummocks and Barnacles compared to the more spatially homogeneous Tile ( $F_{2,66} = 16.78$ ,  $p < 0.001$ ; Figure 5.5c). In contrast, the growth of green MPB was significantly greater on the homogeneous Tile compared to the Barnacles ( $F_{2,66} = 3.12$ ,  $p = 0.05$ ; Figure 5.5d).

### MPB primary production

Gross primary production of the MPB communities showed a significant positive effect with  $p\text{CO}_2$  concentrations, but was not significantly affected by increased temperature or their interaction (Table 5.3; Figure 5.6).

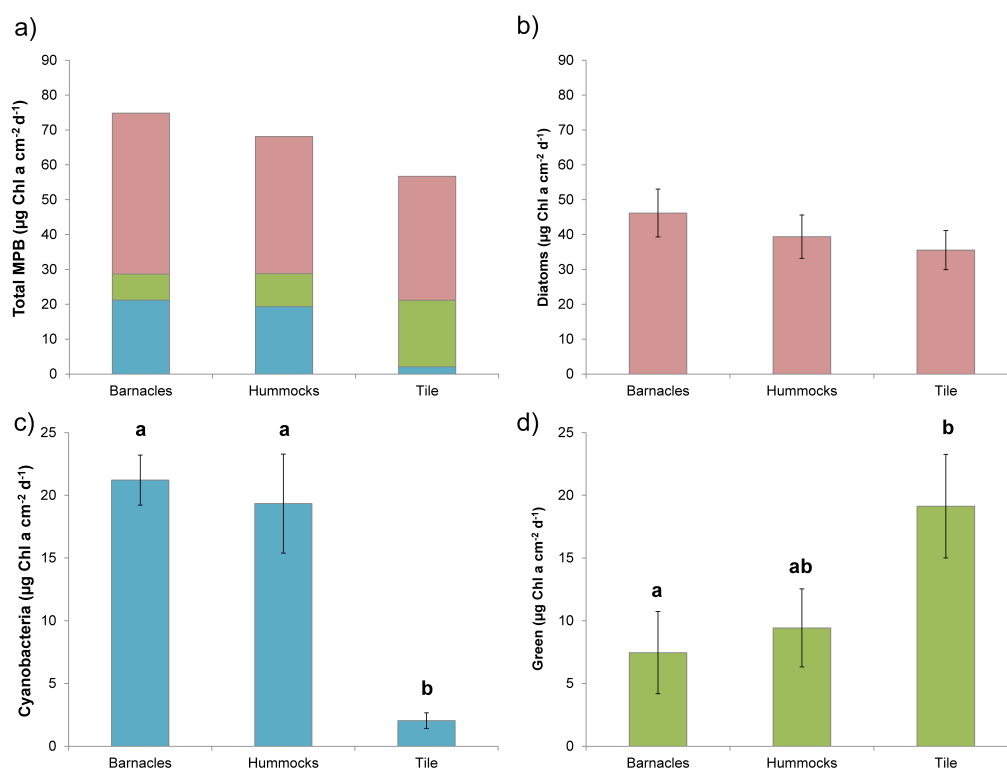


Figure 5.5: Mean ( $\pm$  S.E.) MPB ( $\mu\text{g Chl a cm}^{-2} \text{ d}^{-1}$ ) for a) overview of the relative proportions that the different spectral groups of MPB within the total MPB, b) diatoms, c) cyanobacteria, and d) green,  $n = 6$ . Note different y-axis for panel a-b compared to c-d. Significant differences are highlighted by different lower case letters.

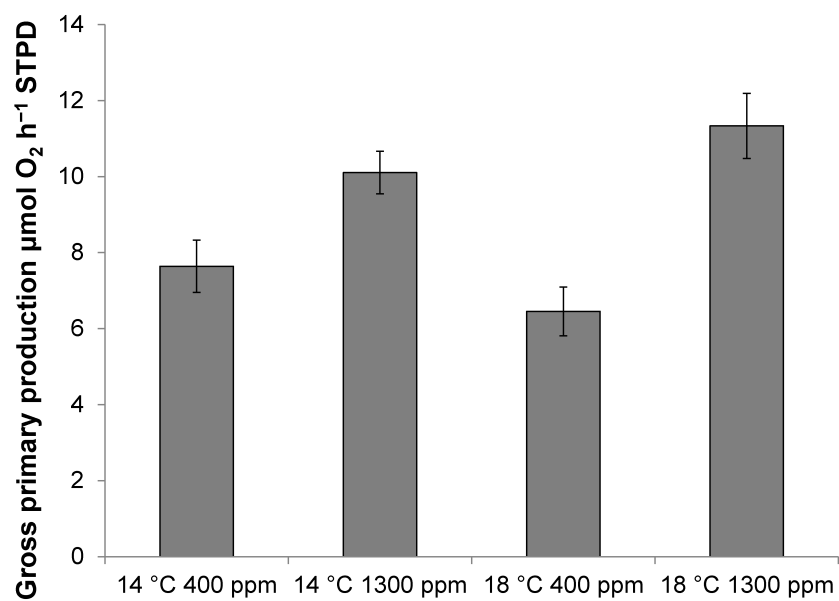


Figure 5.6: Mean ( $\pm$  S.E.) gross primary production of the MPB in the no grazer controls. See Table 5.3 for statistical differences.

Table 5.3: Two-way factorial ANOVA on the effects of  $p\text{CO}_2$  (400 ppm and 1300 ppm) and temperature (14 °C and 18 °C), with temporal block (random effect), on the photophysiology, growth and gross primary production (GPP) of the MPB in the no grazer controls. For factors, degrees of freedom ( $df$ ),  $F$ -values and significance levels are provided, with  $df$  and mean square (MS) values provided for the residuals. Significant tests are shown in bold. \*\*\*  $p < 0.001$ ; \*\*  $0.001 < p < 0.01$ ; \*  $0.01 < p \leq 0.05$ . Results for Tukey HSD *post-hoc* tests are presented below the table.

Factor	$df$	Photophysiology				MPB growth			Primary production
		$\alpha\text{ETR}$	$E_k$	$\text{ETR}_m$	Total MPB	Diatoms	Green	Cyanobacteria	
Block	1	0.18	2.74	<b>7.98 *</b>	0.56	2.96	0.17	<b>22.66 ***</b>	1.01
$p\text{CO}_2$ (OA)	1	0.33	<b>4.71 *</b>	<b>8.98 **</b>	0.30	0.46	0.04	0.41	<b>27.92 ***</b>
Temperature (T)	1	0.25	1.22	<b>5.29 *</b>	<b>4.41 *</b>	<b>6.26 *</b>	0.08	2.35	0.001
OA x T	1	0.01	0.20	0.06	0.16	3.15	1.87	0.45	3.01
Residuals (MS)	19	0.001	387.40	6.44	301.90	191.70	127.50	10.60	2.90
Tukey's <i>post-hoc</i> tests:									
Block		–	–	2 > 1	–	–	–	1 > 2	–
$p\text{CO}_2$ (OA)		–	1300 > 400	1300 > 400	–	–	–	–	1300 > 400
Temperature (T)		–	–	14 > 18	18 > 14	18 > 14	–	–	–
OA x T		–	–	–	–	–	–	–	–

### 5.3.2 Direct responses of grazing assemblage to ocean warming and acidification

#### Community (consumer) respiration

The mean oxygen uptake rate (as a proxy of metabolic rate) of the monoculture consumer communities were all significantly elevated by increased temperature, but were not significantly affected by either elevated  $p\text{CO}_2$  or their interaction (Table 5.4; Figure 5.7a-c). The polyculture, however, demonstrated a significant interaction between  $p\text{CO}_2$  conditions and temperature for mean metabolic rate (Table 5.4), with elevated  $p\text{CO}_2$  conditions in ambient temperature causing a negative trend compared to the control, but a positive trend in the increased temperature treatments (Table 5.4; Figure 5.7d).

Table 5.4: Two-way factorial ANOVA on the effects of  $p\text{CO}_2$  (400 ppm and 1300 ppm) and temperature (14 °C and 18 °C), with temporal block (random effect), on the metabolic rates of the monoculture communities - *P. vulgata*, *G. umbilicalis* and *L. littorea* - and the polyculture (PGL). For factors, degrees of freedom (*df*), *F*-values and significance levels are provided, with *df* and mean square (MS) values provided for the residuals. Significant tests are shown in bold. \*\*\*  $p < 0.001$ ; \*\*  $0.001 < p < 0.01$ ; \*  $0.01 < p \leq 0.05$ . Results for Tukey HSD *post-hoc* tests are presented below the table.

Factor	<i>df</i>	Metabolic rates			
		<i>P. vulgata</i>	<i>G. umbilicalis</i>	<i>L. littorea</i>	PGL
Block	1	4.27	0.66	0.3	3.67
$p\text{CO}_2$ (OA)	1	0.024	0.4	0.53	0.29
Temperature (T)	1	<b>6.81 *</b>	<b>22.4 ***</b>	<b>5.73 *</b>	<b>32.33 ***</b>
OA x T	1	0.54	1.89	0.11	<b>5.45 *</b>
Residuals (MS)	19	91	49.5	112	42.9
Tukey's <i>post-hoc</i> tests:					
Block		–	–	–	–
$p\text{CO}_2$ (OA)		–	–	–	–
Temperature (T)		18 > 14	18 > 14	18 > 14	18 > 14
OA x T		–	–	–	1300:18 = 400:18 > 400:14 = 1300:14

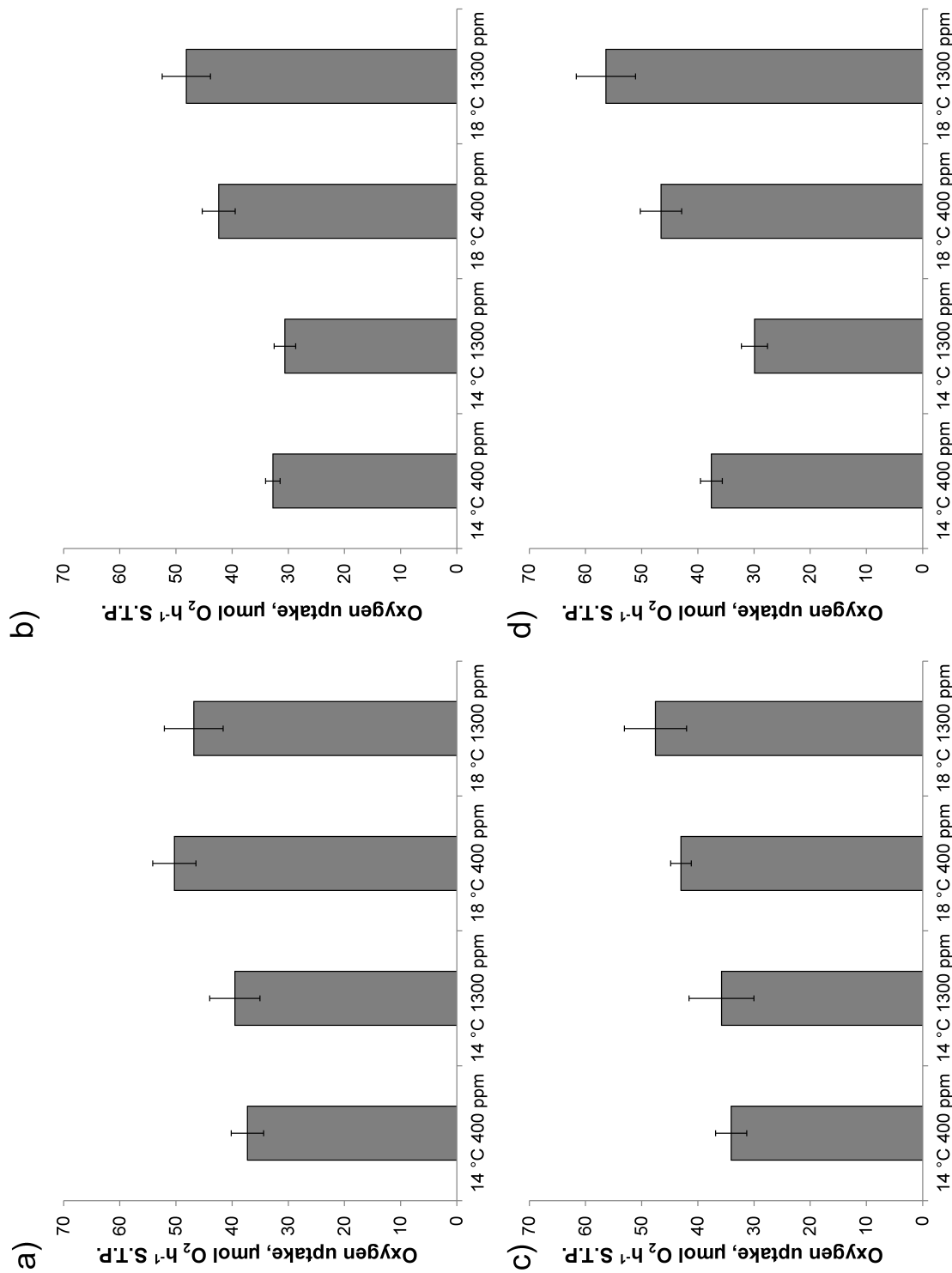


Figure 5.7: Mean ( $\pm$  S.E.) consumer community metabolic rate (oxygen uptake,  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$  S.T.P.) for a) *P. vulgata* monoculture, b) *G. umbilicalis* monoculture, c) *L. littorea* monoculture, and d) the polyculture of all three species,  $n = 6$ . See Table 5.4 for significant differences.



### 5.3.3 Trophic interactions: Net effects of the grazing assemblages

#### ***Patella vulgata***

The net effect (where a positive value represents a reduction in MPB biomass) of *P. vulgata* on the MPB was significantly enhanced by both elevated  $p\text{CO}_2$  conditions and temperature, but with no significant interaction (Table 5.5; Figure 5.8a). At ambient temperatures *P. vulgata* caused a significant negative net effect on the green MPB (Table 5.5, Figure 5.8b), i.e. increased relative to the no grazer controls. The net effect of *P. vulgata* on the diatoms in the MPB was significantly greater in elevated  $p\text{CO}_2$  conditions (Table 5.5; Figure 5.8b).

#### ***Gibbula umbilicalis***

The net effect of *G. umbilicalis* on the MPB was significantly elevated by increased  $p\text{CO}_2$  conditions, but not significantly affected by either increased temperature or their interaction (Table 5.5; Figure 5.8c). Despite not being statistically significantly different, there was a tendency towards an increased net effect at elevated temperatures (Figure 5.8c). The net effect of *G. umbilicalis* on the diatoms within the MPB demonstrated an increased response to elevated  $p\text{CO}_2$  conditions (Table 5.5; Figure 5.8d). The response of the green MPB demonstrated a significant interaction between  $p\text{CO}_2$  conditions and temperature (Table 5.5; Figure 5.8d), whereby the ambient temperature/elevated  $p\text{CO}_2$  concentration and increased temperature/ambient  $p\text{CO}_2$  concentration treatments significantly increased the net effect of *G. umbilicalis* on green MPB relative to both the control conditions, and the combination of both elevated  $p\text{CO}_2$  and temperature (Table 5.5; Figure 5.8d).

#### ***Littorina littorea***

The net effect of *L. littorea* on the MPB was significantly enhanced by both elevated  $p\text{CO}_2$  conditions and increased temperature, but not their interaction (Table 5.5; Figure 5.8e). *L. littorea* also had a tendency for a greater net effect on cyanobacteria with increased temperature (Table 5.5, Figure 5.8f). The net effect by *L. littorea* on diatoms showed a significant interaction between elevated  $p\text{CO}_2$  and temperature, with the combination of elevated  $p\text{CO}_2$  conditions and temperature having a significantly greater net effect compared to all the other treatments (Table 5.5; Figure 5.8f). The net effect of *L. littorea* on the green MPB followed the same pattern as for *G. umbilicalis*, whereby the ambient temperature/elevated  $p\text{CO}_2$  concentration and increased temperature/ambient  $p\text{CO}_2$  concentration treatments significantly increased the net effect of *L. littorea* on green MPB relative to both the control conditions, and the combination of both elevated  $p\text{CO}_2$  and temperature (Table 5.5; Figure 5.8f).

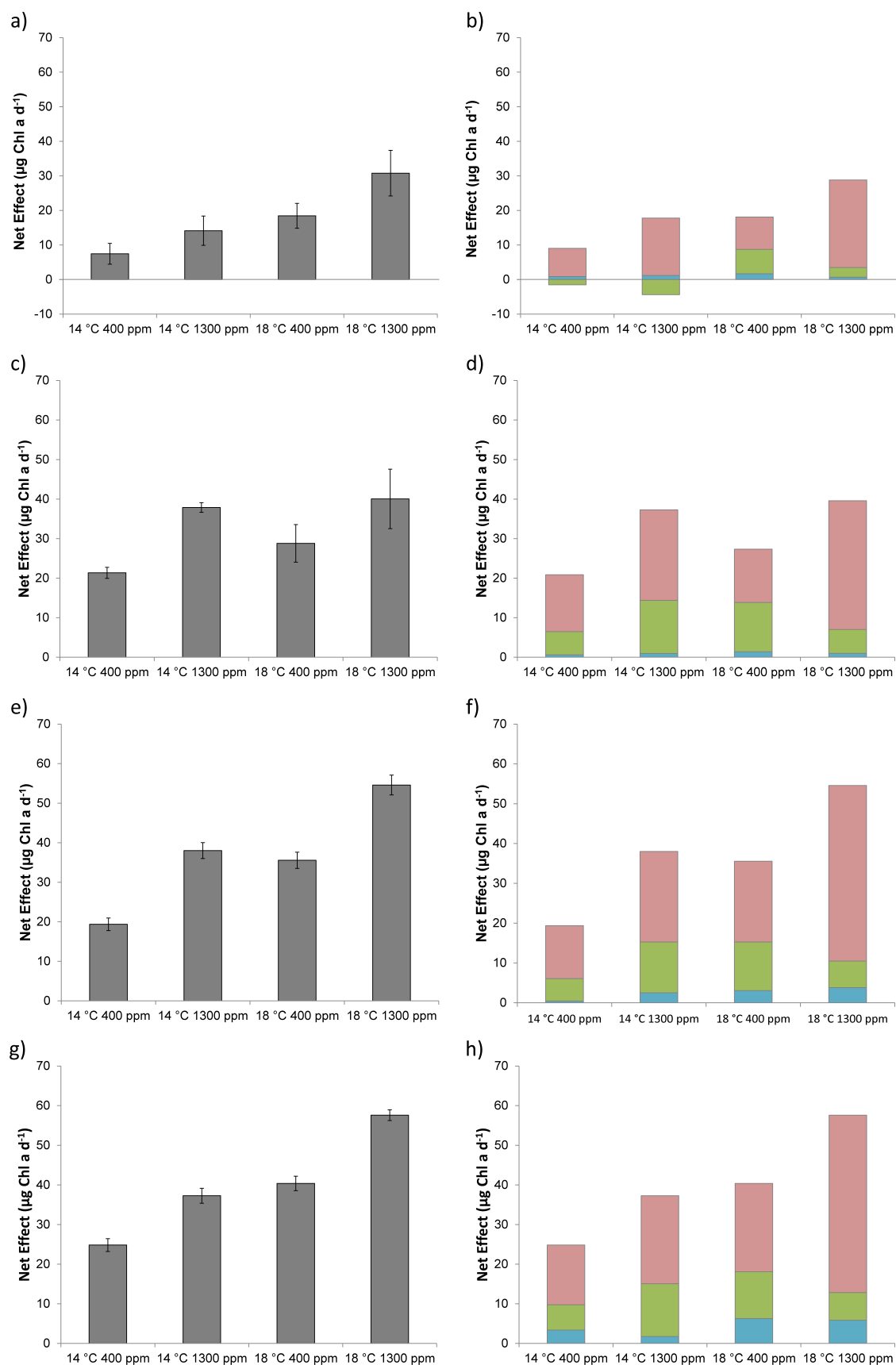


Figure 5.8: Panels a, c, e and g - Mean ( $\pm$  S.E) net effect ( $\mu\text{g Chl a d}^{-1}$ ) of a) *P. vulgata* monoculture, c) *G. umbicalis* monoculture, e) *L. littorea* monoculture, and g) the polyculture on the total MPB. Panels b, d, f and h - Mean net effect of b) *P. vulgata* monoculture, d) *G. umbicalis* monoculture, f) *L. littorea* monoculture, and h) the polyculture in terms of their effect on cyanobacteria (solid blue, bottom group), green (solid green, middle group) and diatoms (solid pink, top group) MPB. For clarity, error bars were omitted from b, d, f and h for significant differences see Table 5.5.

Table 5.5: Two-way factorial ANOVA on the effects of  $p\text{CO}_2$  (400 ppm and 1300 ppm) and temperature (14 °C and 18 °C) with temporal block (random effect), on the net effect of the monoculture communities - *P. vulgata*, *G. umbilicalis* and *L. littorea* - and the polyculture community on the MPB in terms of total net effect, cyanobacteria (Cyano), green and diatom MPB. Temporal blocks for the cyanobacteria, green and diatoms were pooled due to the non-significant block effects for total net effect. For factors, degrees of freedom (*df*), *F*-values and significance levels are provided, with *df* (19 for total, and 20 for different MPB types) and mean square (MS) values provided for the residuals. Significant tests are shown in bold. \*\*\*  $p < 0.001$ ; \*\*  $0.001 < p < 0.01$ ; \*  $0.01 < p \leq 0.05$ . Results for Tukey HSD *post-hoc* tests are presented below the table.

Factor	<i>df</i>	Net effect - <i>P. vulgata</i>				Net effect - <i>G. umbilicalis</i>			
		Total	Diatoms	Green	Cyano	Total	Diatoms	Green	Cyano
Block	1	2.95	–	–	–	1.38	–	–	–
$p\text{CO}_2$ (OA)	1	<b>4.76 *</b>	<b>8.29 **</b>	1.29	0.52	<b>9.48 **</b>	<b>11.35 **</b>	0.65	0.24
Temperature (T)	1	<b>10.04 **</b>	0.26	<b>6.35 *</b>	0.7	1.14	1.13	0.29	0.68
OA x T	1	0.42	0.39	0.05	0.03	0.34	1.69	<b>98.73 **</b>	0.85
Residuals (MS)	19 (20)	133.80	108.10	58.70	8.24	121.90	100.80	3.00	6.14
Tukey's <i>post-hoc</i> tests:									
Block	–	–	–	–	–	–	–	–	–
$p\text{CO}_2$ (OA)	1300 > 400	–	1300 > 400	–	–	1300 > 400	1300 > 400	–	–
Temperature (T)	18 > 14	–	–	18 > 14	–	–	–	–	–
OA x T	–	–	–	–	–	–	–	1300:14 = 400:18 > 400:14 = 1300:18	–

Table 5.5 continued.

Net effect - <i>L. littorea</i>					Net effect – Polyculture				
Factor	df	Total	Diatoms	Green	Cyano	Total	Diatoms	Green	Cyano
Block	1	0.01	–	–	–	<b>6.56 *</b>	<b>5.87 *</b>	<b>6.95 *</b>	0.09
pCO <sub>2</sub> (OA)	1	<b>79.11 ***</b>	<b>222.5 ***</b>	0.58	1.71	<b>99.22 ***</b>	<b>143.81 ***</b>	<b>7.61 *</b>	2.82
Temperature (T)	1	<b>59.79 ***</b>	<b>161.3 ***</b>	0.07	3.18	<b>145.09 ***</b>	<b>144.69 ***</b>	1.43	<b>31.92 ***</b>
OA x T	1	0.01	<b>41.8 ***</b>	<b>39.08 ***</b>	0.36	2.55	<b>38.46 ***</b>	<b>237.70 ***</b>	0.89
Residuals (MS)	19 (20)	26.90	7.50	6.19	7.19	13.3	9.20	0.87	2.33
Tukey's post-hoc tests:									
Block	–	–	–	–	–	1 > 2	1 > 2	1 > 2	–
pCO <sub>2</sub> (OA)	1300 > 400	1300 > 400	1300 > 400	–	–	1300 > 400	1300 > 400	1300 > 400	–
Temperature (T)	18 > 14	18 > 14	18 > 14	–	–	18 > 14	18 > 14	–	18 > 14
OA x T	–	–	1300:18 > all	1300:14 = 400:18 400:14 = 1300:18	–	–	1300:18 > 400:18 1300:14 > 400:14 = 1300:18	1300:14 = 400:18 400:14 = 1300:18	–

### Polyculture community

While significant block effects were observed for the net effect of the polyculture on the MPB, green MPB and diatoms (Table 5.5), due to the first temporal block having a greater net effect by 4, 1 and 3  $\mu\text{g Chl a d}^{-1}$  respectively, this block effect was consistent across treatments, therefore the temporal blocks were pooled. The polyculture demonstrated a significantly greater net effect with both elevated  $p\text{CO}_2$  conditions and increased temperature, with the net effect greater when the drivers were combined (Table 5.5; Figure 5.8g).

The net effects of the polyculture on the diatoms demonstrated a significant interaction between temperature and  $p\text{CO}_2$  conditions. This interaction was due to temperature having an increased net effect in the elevated  $p\text{CO}_2$  conditions relative to the control temperature treatments (Table 5.5; Figure 5.8h). Additionally, the polyculture had an increased net effect at increased temperature and elevated  $p\text{CO}_2$  conditions (separately), with the net effect greatest when the drivers were combined (Table 5.5; Figure 5.8h).

Net effects of the polyculture on the green MPB also demonstrated a significant interaction between  $p\text{CO}_2$  conditions and temperature. The net effect of the polyculture (on green MPB) significantly increased in the ambient temperature/elevated  $p\text{CO}_2$  concentration and increased temperature/ambient  $p\text{CO}_2$  concentration treatments, relative to both the control conditions, and the combination of both elevated  $p\text{CO}_2$  and temperature (Table 5.5; Figure 5.8h). The net effect of the polyculture on cyanobacteria showed a significantly greater net effect with increased temperature (Table 5.5; Figure 5.8h).

#### 5.3.4 Testing diversity effects

### Polyculture vs. best-performing species

Based on the net effects of the monoculture species, the identity of the best performing species changed between the ambient and elevated temperatures (identity  $\times$  temperature;  $F_{3,79} = 3.01$ ,  $p < 0.05$ ). In the ambient temperatures, the net effects of *G. umbilicalis* and *L. littorea* were both significantly greater than *P. vulgata*, but they did not significantly differ from one another (Figure 5.8; Tukey HSD *post-hoc*,  $p < 0.05$ ). In the elevated temperatures, however, *L. littorea* was the best performing species, having a significantly greater net effect on the MPB communities compared to both *G. umbilicalis* and *P. vulgata*.

When comparing the polyculture with the best performing species, no transgressive overyielding was observed, meaning that the polyculture did not exceed the best performing species in any of the experimental treatments (Tukey HSD *post-hoc* tests, all  $p > 0.94$ ).

### Polyculture vs. the mean (additive) monoculture response

The polyculture demonstrated a significantly greater resource utilisation than expected from the monocultures (i.e. the net effects were increased relative to the null additive model) in all treatments (*T*-test, all  $p < 0.05$ ; Figure 5.9a). It was also found that these net effects were not a uniform increase across the different resource types (diatoms, green and cyanobacteria MPB). Instead it was found that the polyculture had a greater net effect (than expected) on the diatoms in elevated temperatures, but not control temperatures

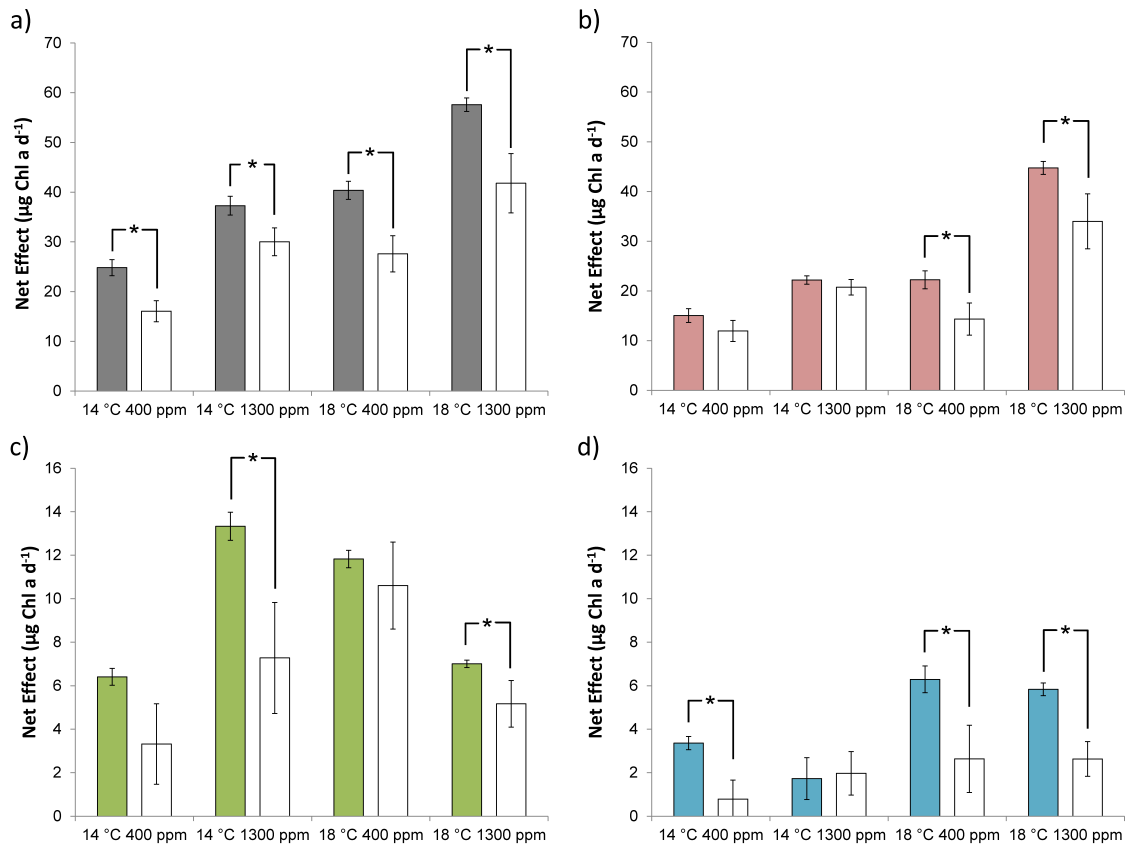


Figure 5.9: Mean ( $\pm$  S.E.) net effect ( $\mu\text{g Chl a d}^{-1}$ ) of the polyculture - a) total MPB (solid grey), b) diatoms (solid pink), c) green (solid green), and d) cyanobacteria (solid blue) - compared to the additive net effect of the monoculture (open bars). Significant differences between the polyculture and the additive net effect (i.e. non-transgressive overyielding) are indicated by an asterisk.

(Figure 5.9b). The green MPB was utilised non-additively in the polyculture when exposed to elevated  $p\text{CO}_2$  treatments, regardless of temperature (Figure 5.9c). Finally, Cyanobacteria were utilised more extensively than predicted in the ambient  $p\text{CO}_2$ /temperature treatment, as well as the elevated temperature treatments (Figure 5.9c). However, the net effect of the polyculture on the cyanobacteria in the elevated  $p\text{CO}_2$  conditions/ambient temperature did not change from the monocultures (Figure 5.9c).

### Substrate utilisation

The three species did not demonstrate any clear patterns of their substrate utilisation being altered by environmental conditions, or strong indications of niche partitioning (Figure D.1).

### Primary production

Environmental conditions had a strong effect on the gross primary production of the remaining MPB communities following grazing from the three monocultures and polyculture (Table 5.6; Figure 5.10). The MPB communities grazed by *P. vulgata* demonstrated the highest GPP when compared to the other treatments (Figure 5.10). For the *P. vulgata* monoculture, increased temperature had a negative effect on the GPP of the MPB, with

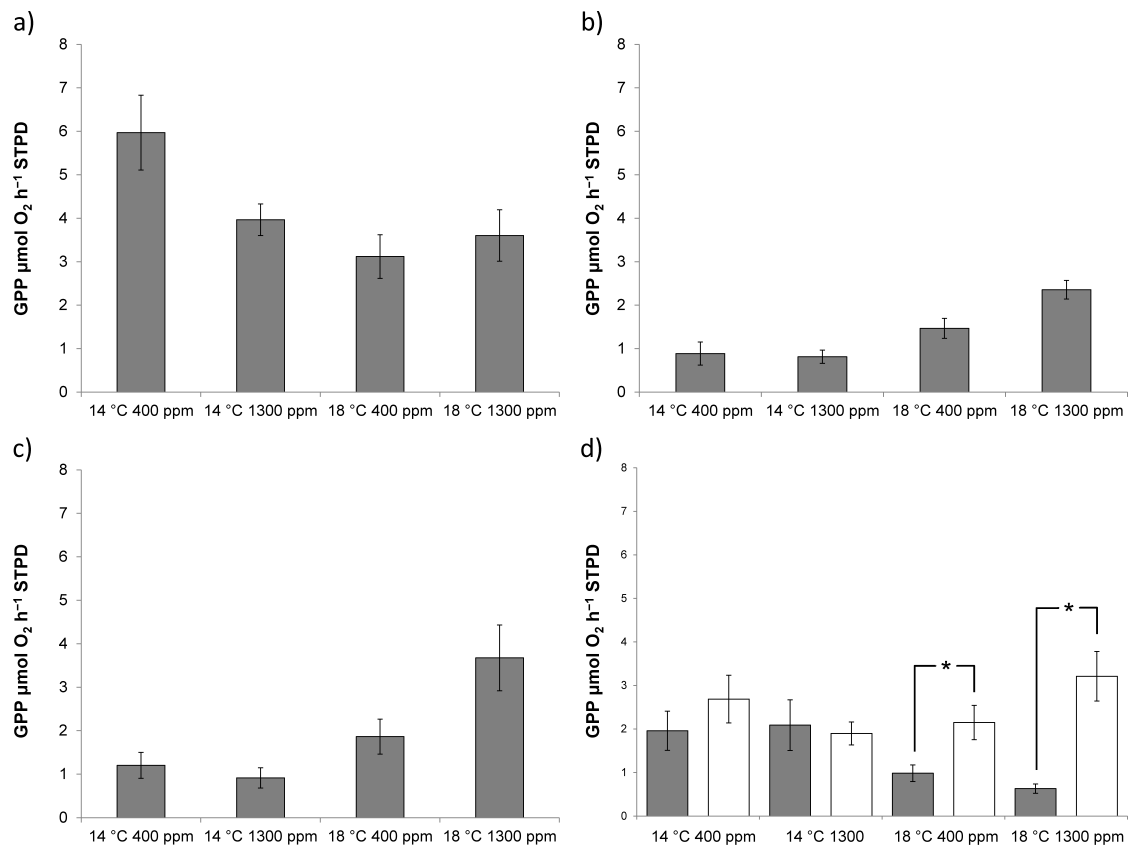


Figure 5.10: Mean ( $\pm$  S.E.) gross primary production (GPP) of the MPB in a) *P. vulgata* monoculture, b) *G. umbilicalis* monoculture, c) *L. littorea* monoculture, and d) the polyculture (solid grey bars) compared to the null additive model (open bars). See Table 5.6 for the statistical differences for the panels a-c. Statistical differences between environmental conditions for the polyculture community (panel d) are also in Table 5.6, however, statistical differences between the polyculture and the additive null model are indicated here with an asterisk.

elevated  $p\text{CO}_2$  conditions not having a significant effect (Table 5.6; Figure 5.10a). In contrast, the MPB responses in both the *G. umbilicalis* and *L. littorea* monocultures showed a significant interaction between  $p\text{CO}_2$  and temperature, due to elevated  $p\text{CO}_2$  conditions only increasing GPP during the higher temperature treatment (Table 5.6; Figure 5.10b and c). In the polyculture treatment, temperature significantly reduced the productivity of the MPB, but appeared unaffected by elevated  $p\text{CO}_2$  conditions (Table 5.6; Figure 5.10d). When comparing the polyculture to the null additive model, the MPB communities from the polyculture treatment maintained similar levels of GPP in the ambient temperature conditions, however, demonstrated a significantly reduced GPP (relative to the additive model) in the increased temperature conditions.

Table 5.6: Two-way factorial ANOVA on the effects of pCO<sub>2</sub> (400 ppm and 1300 ppm) and temperature (14 °C and 18 °C), with temporal block (random effect), on the primary productivity of the MPB following the net effect of the monocultures and polyculture. For factors, degrees of freedom (*df*), *F*-values and significance levels are provided, with *df* and mean square (MS) values provided for the residuals. Significant tests are shown in bold. \*\*\* *p* < 0.001; \*\* 0.001 < *p* < 0.01; \* 0.01 < *p* ≤ 0.05. Results for Tukey HSD *post-hoc* tests are presented below the table.

Factor	df	MPB gross primary productivity				MPB standing stock following polyculture net effect			
		<i>P. vulgata</i>	<i>G. umbilicalis</i>	<i>L. littorea</i>	Polyculture	Total	Cyano	Green	Diatoms
Block	1	0.02	0.40	0.65	0.19	<b>7.04 *</b>	0.12	<b>7.65 *</b>	<b>5.89 *</b>
pCO <sub>2</sub> (OA)	1	1.49	3.36	2.59	0.08	0.08	0.01	0.49	0.03
Temperature (T)	1	<b>6.65 *</b>	<b>22.63 ***</b>	<b>13.10 **</b>	<b>9.64 **</b>	1.38	<b>5.26 *</b>	<b>4.79 *</b>	0.1
OA x T	1	3.99	<b>4.64 *</b>	<b>4.93 *</b>	0.38	2.5	<b>5.10 *</b>	0.96	0.26
Residuals (MS)	19	2.33	0.30	1.34	0.92	0.85	0.01	0.01	0.07
Tukey's <i>post-hoc</i> tests:									
Block	–	–	–	–	–	2 > 1	–	2 > 1	2 > 1
pCO <sub>2</sub> (OA)	–	–	–	–	–	–	–	–	–
Temperature (T)	14 > 18	18 > 14	18 > 14	18 > 14	14 > 18	–	14 > 18	14 > 18	–
OA x T	–	1300:18 > all	1300:18 > all	1300:18 > all	–	–	400:14 = 400:18 1300:14 > 1300:18	–	–



### MPB standing stock

The MPB standing stock (i.e. the remaining MPB biomass following grazing) was quantified, in order to see whether the observed reduction in the GPP following grazing from the polyculture was associated with less MPB biomass, or less MPB biomass of a specific resource type. Therefore, comparisons were made between the polyculture, and the mean (additive) expectation of the monocultures. It was found that following the non-transgressive overyielding, the MPB standing stock was significantly reduced non-additively to the monocultures ( $T$ -test, all  $p < 0.05$ ; Figure 5.11a). Interestingly, visual comparisons suggest that the differences were greater in the elevated temperature treatments (Figure 5.11a).

When investigating just the polyculture responses, we found no significant differences in the amount of remaining MPB biomass between environmental treatments (Table 5.6). However, we did find differences between the specific resource types. The remaining Green MPB was significantly reduced in the increased temperature treatments relative to the ambient temperature treatments (Table 5.6; Figure 5.11b). Cyanobacteria was also reduced by increased temperature, but only when combined with the elevated  $p\text{CO}_2$  conditions (Table 5.6; Figure 5.11b). The standing stock of diatoms was, however, unaffected by the environmental treatments in the polyculture. It must be noted, that block effects were observed for the total, green and diatoms MPB (see Table 5.6), but they were pooled across the temporal blocks due to the differences being consistent across all the treatments.

## 5.4 DISCUSSION

In general, increased grazer biodiversity appeared to influence several ecosystem processes (consumer net effect/resource utilisation, gross primary production, and standing stock) and hence, either promote or at least sustain ecosystem multi-functionality (i.e. the positive functioning of the ecosystem, across several ecosystem processes). However, the ability to sustain multi-functionality at the elevated temperatures resulted in a trade-off between ecosystem processes, meaning that it was no longer possible to sustain all the processes. This trade-off was exhibited at increased temperatures where gross primary production of the MPB community was reduced, in order to maintain increased resource utilisation (i.e. non-transgressive overyielding by the consumers). This suggests that the trophic interactions between the grazing assemblage and the MPB communities will increasingly shift towards more extreme heterotrophy in warmer oceans.

Although the direct responses of the consumers (i.e. oxygen uptake) appeared relatively similar, we observed some differences in the net effects that they had on the MPB communities, including the best performing species (based on the same metabolic biomass) changing at increased temperature. The consumers appeared to be contributing differently to ecosystem processes (e.g. net effects, GPP), suggesting that the diversity effects was associated with species identity effects, rather than an overall richness effect (i.e. it mattered which species, rather than just the number of species). It is suggested that the positive diversity effects observed could include facilitation<sup>2</sup> (e.g. [Cardinale \*et al.\*, 2002](#)),

<sup>2</sup> Facilitation - "Facilitative, or positive, interactions are encounters between organisms that benefit at least one of the participants and cause harm to neither." Taken from [Stachowicz \[2001\]](#).

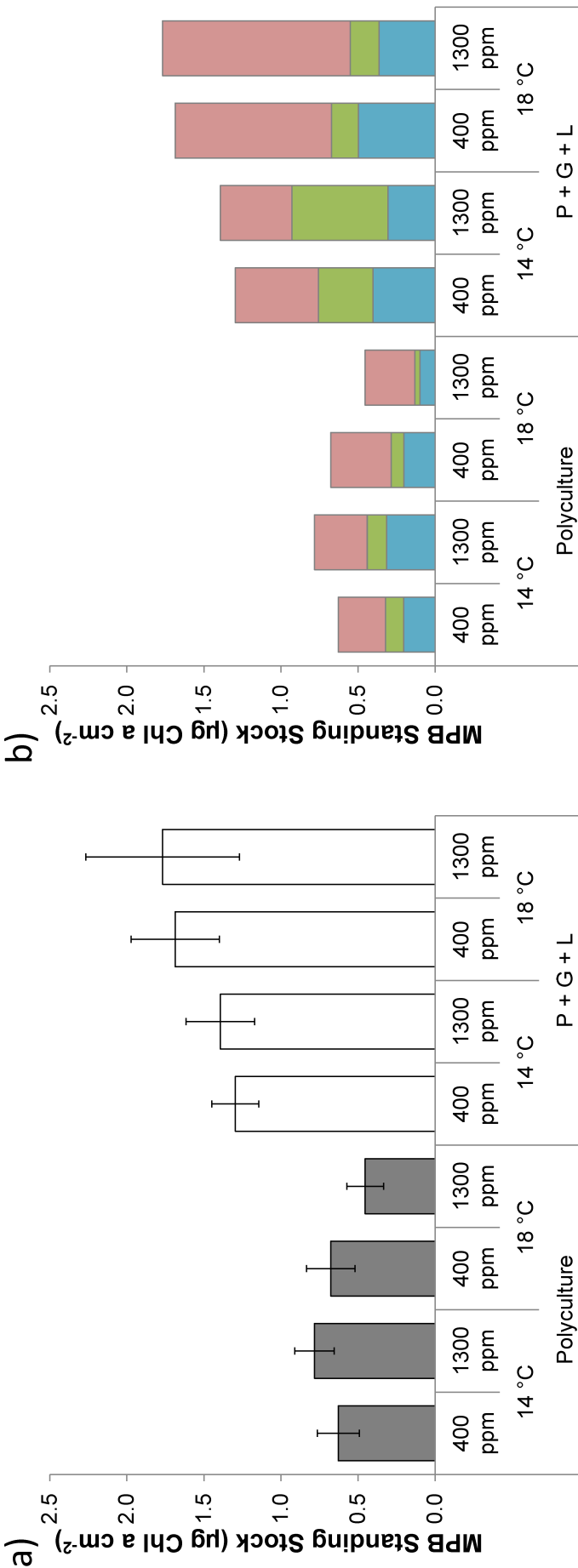


Figure 5.11: Mean ( $\pm$  S.E.) MPB standing stock ( $\mu\text{g Chl a cm}^{-2}$ ) following a) the net effects of the polyculture grazing assemblage (Polyculture, solid grey bars) or the additive mean monoclulture response (P + G + L, solid white bars). Panel B depicts the same MPB standing stock in terms of the remaining cyanobacteria (solid blue, bottom group), green (solid green, middle group) and diatoms (solid pink, top group) MPB. For clarity, error bars were omitted from b.

but is more likely associated with resource partitioning (e.g. [Cardinale, 2011](#)). Overall, this key finding highlights the need to consider the multifunctionality of ecosystems across a range of environmental conditions in order to achieve a greater understanding of how future climate change may alter biodiversity-ecosystem functioning relationships.

#### 5.4.1 *Direct responses of the MPB community to ocean warming and acidification*

The photosynthetic parameters of the MPB community (i.e.  $E_k$  and  $ETR_m$ ) responded positively to elevated  $pCO_2$  conditions and negatively to increased temperature.  $E_k$  represents the optimum irradiance level required to balance the temperature-independent photosynthetic energy capture and the temperature-dependent capacity of the photosynthetic system to process this energy ([Falkowski & Raven, 2007](#)). Hence, the increased  $E_k$  with elevated  $pCO_2$  conditions means that an increased maximal amount of light could be processed (while still avoiding photoinhibition, [Raven & Geider, 1988](#)), likely stimulated by the increased maximal photosynthetic efficiency (e.g. [Suárez-Álvarez et al., 2012](#)). Similarly, the reverse (reduced maximal light-utilisation) could be said with increased temperature.

Interestingly, despite the reduced photosynthetic efficiency observed with increased temperature, we actually observed an increased propagation of MPB. Contrastingly, the positive photosynthetic efficiency with elevated  $pCO_2$  conditions resulted in no change in growth. Growth limitation by  $CO_2$  in marine diatoms, green algae and cyanobacteria has been reported from both field and lab work ([Riebesell et al., 1993](#); [Hutchins et al., 2007](#); [Riebesell et al., 2007](#)), and hence, it must suggest that our MPB community is not  $CO_2$ -limited, perhaps due to the presence of carbon-concentrating mechanisms (CCM; [Gior-dano et al., 2005](#); [Raven et al., 2011](#)) or because limitations were due to other aspects (e.g. nutrient-limitation).

#### 5.4.2 *Direct responses of grazing assemblage to ocean warming and acidification*

The three consumers utilised here are all grazing gastropods belonging to the same 'functional group' ([Simberloff & Dayan, 1991](#); [Wilson, 1999](#)), however, they are expected to affect the MPB communities differently (i.e. identity effects), due to differences in their dietary preferences ([Steneck & Watling, 1982](#); [Hawkins & Hartnoll, 1983](#); [Hawkins et al., 1989](#)) and modes of feeding ([Lubchenco, 1978](#); [Watson & Norton, 1985](#)). In this study, the monoculture grazing assemblages did demonstrate some differences in their overall net effects on the MPB communities, including differences in their net effects on the different resource types (diatoms, green, cyanobacteria). However, while these can be described as 'identity effects', they were not striking differences.

The experimental design used in this study meant that our grazing assemblages had similar metabolic body mass, and should therefore demonstrate similar interactions within food webs ([Schmitz & Price, 2011](#); [Atkins et al., 2015](#)), that scale with temperature (*sensu* 'metabolic theory of ecology', [Brown et al., 2004](#)). As expected, the two best performers (in the ambient temperature treatments) - *G. umbilicalis* and *L. littorea* - demonstrated similar net effects on the MPB biomass. During the elevated temperature treatments, *L. littorea* became the best performing species, and the net effects of *L. littorea* did indeed scale with temperature (i.e. consumption/net effect increased as metabolic rate

increased). Although *G. umbilicalis* did not display a significant temperature effect, a tendency for having a greater net effect during increased temperatures was also demonstrated.

Previous work has demonstrated vast changes in community structure following the removal of limpets from natural systems (Jones, 1948; Southward & Southward, 1978), with the suggestion that these species could be considered a 'keystone' species (*sensu* Paine, 1966). Therefore, it was interesting that the *P. vulgata* monoculture actually demonstrated a reduced net effect (relative to the other grazers) due to a net positive effect on the green MPB in the ambient temperatures. This was likely through nutrient release or excretion promoting MPB growth (e.g. Sherr *et al.*, 1986). Should this process occur in natural settings, then *P. vulgata* may not be functionally redundant due to the positive effect that they have on the ecosystem processes of MPB biomass and (presumably) secondary production. It is extremely likely, however, that this is an experimental artefact (or tank effect), and it is suggested that such a localised accumulation of nutrients/faeces would be unlikely to occur under natural conditions, making it likely that the net effect of *P. vulgata* was underestimated.

It was demonstrated that the elevated  $p\text{CO}_2$  conditions did not significantly affect the metabolic rate of the monoculture grazing assemblages. Hence, the net effect of the monoculture grazing assemblages on the MPB communities would be expected to be comparable to the ambient  $p\text{CO}_2$  conditions of the same temperature. Instead, we observed an increased net effect that was beyond what might be expected based on metabolic biomass alone. This would suggest that the elevated  $p\text{CO}_2$  conditions are influencing the food quality of the MPB, forcing the grazing assemblages to initiate compensatory feeding (e.g. Cruz-Rivera & Hay, 2000). Changes in the nutritional quality of the MPB could be associated with the elevated  $p\text{CO}_2$  conditions reducing their fatty acid composition (e.g. diatoms, Rossoll *et al.*, 2012), or changes in their stoichiometry (C:N ratios, van de Waal *et al.*, 2009) reducing their palatability. Another intriguing possibility is that the enhanced grazing in the elevated  $p\text{CO}_2$  conditions could represent a countervailing mechanism that allows the consumer to absorb disturbances to the MPB community through trophic interactions, subsequently promoting ecosystem resistance (e.g. Alsterberg *et al.*, 2013; Ghedini *et al.*, 2015). Such trophic compensation may act well before species loss occurs, but are also typically considered to potentially go unexplored since they would produce no outward change in community structure or function (Ghedini *et al.*, 2015).

#### 5.4.3 Diversity effects - facilitation and niche partitioning

Facilitation and the niche partitioning of resource types and substrates are principal mediators of how biodiversity can influence the rates of resource use that govern the efficiency and productivity of ecosystems (Tilman *et al.*, 1997; Loreau & Hector, 2001; Cardinale *et al.*, 2002; Cardinale, 2011). Here, it was observed that despite no clear identity effects of the monoculture grazing assemblages, the diverse polyculture managed to achieve a non-additive increase in resource utilisation relative to the monocultures, and this was even across all of the environmental conditions. This was exhibited through an increased usage of different resource types, with the magnitude differing with environmental context. Based on the monoculture, it is proposed that *P. vulgata* demonstrated a positive interaction on the polyculture by facilitating MPB growth. As previously discussed, however, this effect by *P. vulgata* is likely a tank effect, but this would also suggest that the net effects

of the polyculture could also have potentially been underestimated. Consequently, the majority of the species richness effects on the increased resource utilisation appeared to be more strongly associated with niche partitioning, i.e. differential usage of resource types.

#### 5.4.4 *Ecosystem functioning of the MPB - Species identity and richness*

Epilithic biofilms are often treated as a single entity for ecological studies, and yet they have their own richness and structure (Jackson *et al.*, 2001) and hence, MPB communities will importantly demonstrate their own diversity-function relationships in response to global environmental change. Previous work has demonstrated that elevated  $p\text{CO}_2$  conditions and temperature can result in shifts in the species structure of the MPB communities, including reduced species diversity of photo-autotrophic species (particularly cyanobacterial species, Witt *et al.*, 2011; Russell *et al.*, 2013), but increases in bacterial diversity and abundance (Lidbury *et al.*, 2012; Kerfahi *et al.*, 2014). Such changes will importantly influence MPB primary production (Russell *et al.*, 2013). The productivity (and structure) of the MPB communities will, however, also be affected by consumption from the grazers. Besides grazing having a negative effect on primary production by causing the loss of photosynthetic tissue mass, the effect of the grazers can also cause community succession in epilithic biofilm communities (Jackson *et al.*, 2001), and may even have positive effects on their formation, such as enhanced growth and production (Huws *et al.*, 2005; Matz & Kjelleberg, 2005).

In this study, the MPB communities were generally less productive compared to the grazer free control, almost certainly due to grazer-induced MPB biomass reductions. However, despite the greater top-down control in the increased temperature treatments, the grazing by both the *G. umbilicalis* and *L. littorea* monocultures actually increased the gross primary production of their MPB communities (relative to the ambient, but still grazed, treatments). Previous studies have demonstrated that grazing can promote the photo-autotrophic aspects of the biofilm by consuming the less productive heterotrophic biofilm canopy (Skov *et al.*, 2010), and it possible that a similar mechanism is being utilised here.

The environmental context of these identity effects would suggest that even should a species appear to demonstrate functional redundancy under particular environmental conditions, they are likely to contribute to additional services under different environmental conditions (Mulder *et al.*, 2001). Hence, while the grazing assemblages presented here do demonstrate an overall complementarity for ecosystem processes (e.g. increased resource utilisation), as the environmental conditions change they do not appear to demonstrate much within-process redundancy, despite being comprised of the same functional group. i.e. each grazer assemblage appeared to promote different ecosystem processes (e.g. more productive MPB relative to biomass). In contrast, a recent study by Perkins *et al.* [2015] found that it was the additive effects of functionally different species that enabled positive ecosystem multifunctionality (displaying within-process redundancy, as well as across-process complementarity), thus suggesting that the responses within and between functional groups will likely differ.

Just as increased temperature strengthened the consumer-driven control of the MPB community in the monocultures, the increased ecosystem functioning of the polyculture (i.e. the increased resource utilisation) on the MPB communities during the increased

temperature treatments resulted in a reduced standing MPB biomass. This is likely associated with the increased sensitivity of heterotrophic metabolism to increased temperature compared to autotrophic metabolism and production (Allen *et al.*, 2005; Lopez-Urrutia *et al.*, 2006; O'Connor *et al.*, 2009). Subsequently, although the gross primary production of the MPB community could be maintained in the ambient temperatures, it was reduced non-additively to the monoculture in the elevated temperatures. This was likely due to the MPB standing stock only being maintained at a similar level across treatments, despite MPB communities displaying an increased growth rate in the elevated temperatures. Hence, it is likely the increased temperature is having some combination of direct effects (reduced MPB photosynthetic efficiency) as well as trophic interactions (enhanced resource utilisation by the consumers) that ultimately results in the observed reduction in MPB primary production. Therefore, this study highlights how maintaining the ecosystem functioning of grazer and MPB communities may require trade-offs between ecosystem processes (primary production, standing stock and resource utilisation), and how environmental conditions could alter the balance between these processes (also see Perkins *et al.*, 2015).

#### 5.4.5 *Summary and conclusions*

The consequences for the ecosystem functioning of the MPB communities under future climate change demonstrate that generally, grazing assemblages from the same functional group can promote ecosystem processes relative to the less diverse monocultures. Despite positive effects of the elevated  $p\text{CO}_2$  conditions on the photosynthesis of the MPB communities, this was not exhibited in MPB growth, and moreover any potential disturbances appeared to strongly controlled by the grazers. More importantly, however, when temperature was increased, this induced a shift towards heterotrophy, resulting in a trade-off in the ecosystem functions of grazer resource utilisation and MPB primary production. Overall, the diversity effects observed here appeared to be more of a complementary contribution of species identity effects, rather than a generic species richness effect, and it is suggested that overall functioning is likely to be contingent on both biodiversity and environmental context. Subsequently, testing the performance of individual species (rather than functional groups) in response to climate change may be required, in order to more reliably assess their contribution to ecosystem functioning. Regardless, this study importantly highlights the need to consider the multifunctionality of ecosystems in response to future climate change, in order to achieve a more realistic understanding of how biodiversity-ecosystem functioning relationships might alter.

## INDIVIDUAL AND POPULATION-LEVEL RESPONSES TO CLIMATE CHANGE

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### Abstract

Ocean acidification, the change in seawater carbonate chemistry associated with increasing levels of atmospheric CO<sub>2</sub>, is predicted to have detrimental effects on many marine organisms and ecological processes. Despite rapidly growing evidence for the direct impacts on individual species, few studies have considered the long-term consequence of ocean acidification, and empirically linked the energetic consequences of the individual to the contemporary demographic processes of the population. Here, we test whether it is possible to scale from the potential impacts on individual physiology and fitness of the banded murex, *Hexaplex trunculus* (Linnaeus, 1758), up to population level demographic processes, in the face of ocean acidification over multiple generations, and whether such long-term chronic exposure will result in any genetic or morphological divergence. This study shows that ocean acidification, against a background of unrestricted gene flow, increases energetic demands of individuals resulting in altered energy allocation, revealed by reductions in shell size and maintenance but increased body and gonad size. When scaled up to the population-level, long-term exposure to ocean acidification reduced population size and altered population demography with evidence of a reduction in the proportion of females in the population and genetic signatures of increased variance in reproductive success among individuals. Such increased variance leads to greater levels of short-term genetic drift that is predicted to oppose adaptation. Importantly, this study shows that even against a background of high gene flow ocean acidification is driving individual and population level changes that will alter eco-evolutionary trajectories.





## 6.1 INTRODUCTION

Marine organisms are capable of tolerating moderate environmental change by regulating their physiology (i.e. acclimatisation) in order to maintain cellular homeostasis (Pörtner *et al.*, 2004). Energy allocation strategies will typically prioritise those functions required for basal maintenance, allocating the remainder towards fitness-related activities, such as tissue production, calcification and reproduction (Kooijman, 2010). Ocean acidification represents a deleterious anthropogenic influence that can affect the vital functions of marine organisms (Feely *et al.*, 2004; Doney *et al.*, 2009). Significant and persistent environmental change (such as ocean acidification) can modulate metabolic activity through changes in energy demand and/or availability (Dahlhoff *et al.*, 2002), and modify how energy is being allocated within an organism (e.g. Stumpp *et al.*, 2012b).

The distribution of energy will primarily be dictated by a heritable set of rules (i.e. natural selection) that maximise fitness under a particular set of environmental conditions (Thor & Dupont, 2015). Parents are capable of positively influencing the response of their offspring to environmental change either genetically, by selection or adaptive evolution (Howells *et al.*, 2012; Lohbeck *et al.*, 2012; Pespenti *et al.*, 2013b) or non-genetically, through molecular phenotypic responses (e.g. epigenetics, Turner, 2009) or maternal investment (Podolsky & Moran, 2006; Allen *et al.*, 2008). However, knowledge on how marine organisms will respond to multi-generational exposure to ocean acidification is scarce (but see Miller *et al.*, 2012; Parker *et al.*, 2012; Schade *et al.*, 2014; Thor & Dupont, 2015), and therefore represents one of the major challenges of ocean acidification research (Riebesell & Gattuso, 2015) - scaling from acclimation to adaptation.

Significant evolutionary responses are expected during ocean acidification (Harvey *et al.*, 2014; Sunday *et al.*, 2014 for reviews), and yet little is known about how the key demographic transitions (that influence population dynamics) will be affected during ocean acidification. This is because past studies have tended to focus on the more immediate responses of individual marine species to ocean acidification (Kelly & Hofmann, 2013). The current knowledge gap is linking these responses up to populations and eventually, whole ecosystems (Dupont & Pörtner, 2013). Establishing the capacity of populations to manage the negative effects of ocean acidification will not only require understanding the physiological response of the individual (as indicated by their energy allocation strategies), but also population-level demographics and genetic structuring, such as gene flow, reproductive success and population size.

Our research seeks to address this knowledge gap by using natural *in-situ* CO<sub>2</sub> seeps which will allow experimental work to simultaneously capture both the individual physiological responses, as well as fitness-related traits, such as reproductive success, over multiple generations of exposure to ocean acidification. Specifically, three pre-established sites (e.g. Johnson *et al.*, 2012; Suggett *et al.*, 2012; Boatta *et al.*, 2013) within the study region were used: one low pH site within the seep system (~pH 7.7; representing carbonate chemistry conditions predicted for 2100 under IPCC RCP 8.5 (IPCC, 2013), and two control sites outside the system (~pH 8.2 representing current ocean conditions; Figure 6.2).

Our study species was the banded murex, *Hexaplex trunculus* (Linnaeus, 1758, Figure 6.1), a muricid gastropod predominantly found throughout the Mediterranean Sea (Poppe

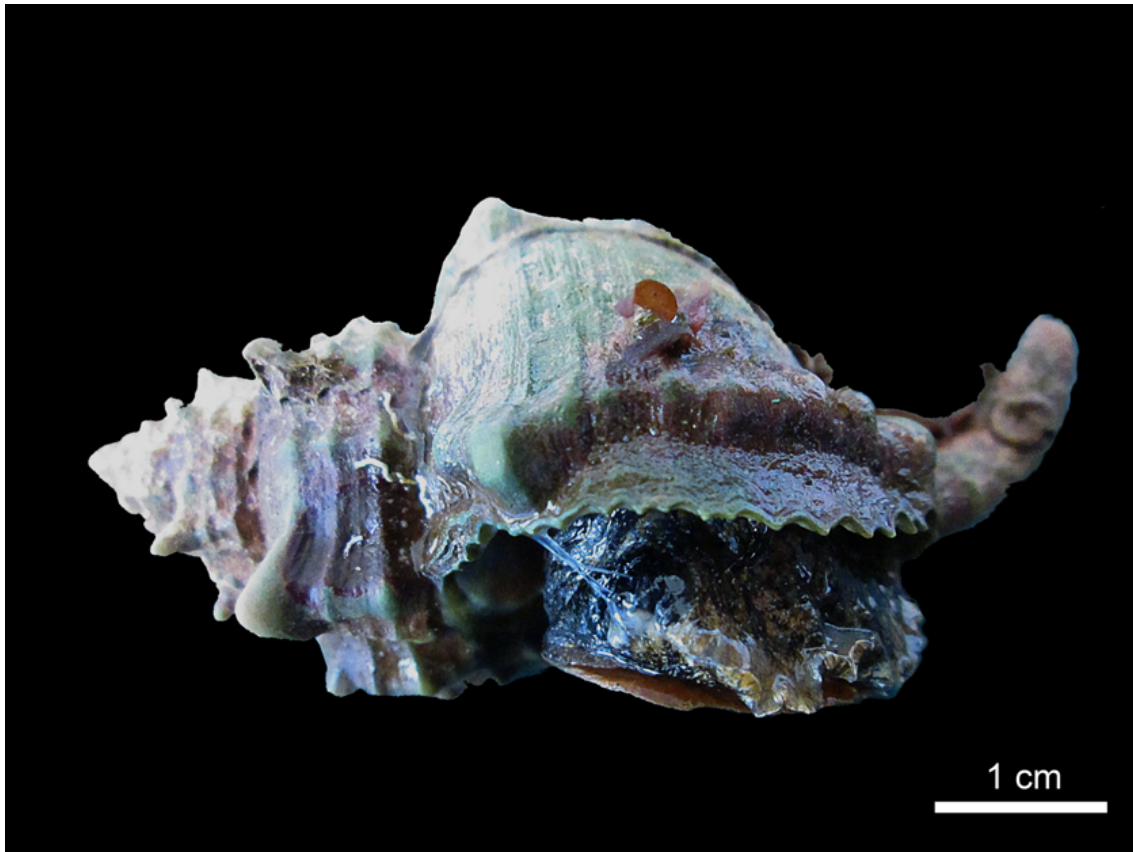


Figure 6.1: Banded murex, *Hexaplex trunculus* (Linnaeus, 1758).

& Goto, 1991; Houart, 2000). It is an important species of commercial interest, being fished for both human consumption as well as for fish bait (Benović, 1997). It is a direct developer (lacks a pelagic larval stage) and both juveniles and adults are highly restricted in mobility, resulting in a limited ability to colonise new areas (Vasconcelos *et al.*, 2004). This limited dispersal capacity (relative to the spatial scale of the seeps site) also means that populations from within the CO<sub>2</sub> seep sites are likely to have been under those novel conditions for multiple generations. Subsequently, this makes them an ideal model organism in which to investigate individual to population-level responses under prolonged (multiple generations), chronic exposure to elevated  $p\text{CO}_2$ . Therefore, the overall aim of this study was to test whether it is possible to scale from the potential impacts on individual physiology and fitness of *H. trunculus*, up to population level demographic processes, in the face of ocean acidification over multiple generations, and whether such long-term chronic exposure will result in any genetic or morphological divergence.

At the individual level we carried out a reciprocal transplant between the Low pH site and one of the Control sites, and measured the metabolic rates of *H. trunculus*. Measuring individuals under the same conditions that they were collected allowed us to ascertain the 'cost of living' in ocean acidification relative to ambient pH conditions, whereas measurements following the reciprocal transplant between pH conditions allowed us to disentangle whether the responses observed were plastic or adaptive responses. The energy allocation strategies of *H. trunculus* from the three sites (Low pH site, and the two control pH sites - termed Control and Reference site; see Figure 6.2) were then tested by measuring somatic tissue mass, gonad size, and shell length and thickness. We hypothesised that ocean acidification would increase the cost of living (metabolic rate), resulting

in trade-offs in the allocation of energy between growth, calcification and reproduction.

For population dynamics, phenotypic and genetic adaptation to ocean acidification were tested by measuring morphological divergence by using geometric morphometrics (shape analysis) of the shells, and between-population differentiation using allele frequencies and molecular markers, as well as population size and sex ratio. In all cases, these comparisons were between the Low pH within the CO<sub>2</sub> seeps system, and the nearby Control and Reference site (see Figure 6.2). We hypothesised that the elevated pCO<sub>2</sub> conditions would act as a barrier to gene flow, resulting in both morphological and genetic divergence, but (when combined with energetic trade-offs at the individual-level) would result in a reduced population size.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Study site

The study area was a CO<sub>2</sub> seep system in Levante Bay (38 ° 25' N, 14° 57' E), which is located on the north-east side of the volcanic island, Vulcano (Italy). Nearby volcanic activity in the bay includes gas vents along an active fault (Frazzetta *et al.*, 1984) releasing approximately 3.6 tonnes of CO<sub>2</sub> per day (Inguaggiato *et al.*, 2012). The acidified water masses creates a pH gradient ranging from 6.8 to 8.2 along the northern shoreline of the bay, due to the predominantly wind-driven currents from the western to north-western sector (Boatta *et al.*, 2013).

Three sites along the natural pH gradient were used (Figure 6.2). All three sites experience stable levels of ambient temperature and salinity, with the mean surface seawater pH progressively increasing with proximity to the CO<sub>2</sub> vents (Table 6.1). The Low pH site represented elevated pCO<sub>2</sub> conditions ( $7.77 \pm 0.03$  pH<sub>NBS</sub>) and was located nearest the seep (250m distance, 38° 25.164'N; 14° 57.704'E). The Control site represented ambient pH conditions ( $8.12 \pm 0.01$  pH<sub>NBS</sub>) over the long-term, albeit with periods of short-term temporal (hours/days) pH variability, and was located further from the seep (400m distance, 38° 25.209'N, 14° 57.804'E). Finally the third site, termed Reference, represented stable, ambient pH conditions ( $8.18 \pm 0.01$  pH<sub>NBS</sub>), and was unaffected by the elevated pCO<sub>2</sub> from the vents. Reference was located farthest from the seep (600m distance, 38° 25.262'N, 14° 57.919'E). These conditions are typical of CO<sub>2</sub> seep study sites (Calosi *et al.*, 2013a).

### 6.2.2 *Hexaplex trunculus* collection

Collections of *H. trunculus* (and subsequent experiments) were carried out over two experimental periods, May 2013 and May 2014. During the first experimental period, individuals used for the reciprocal transplant were collected and deployed in transplantation cages, with additional individuals collected in order to measure geometric morphometrics (shell shape analysis). During the second sampling period individuals were destructively sampled for sex determination, body size (including gonad size) and shell thickness (outer lip). Samples for genetic analysis were collected during both experimental periods in order to test for the temporal stability of any population structuring and/or demographics. More specific descriptions for each experiment are outlined below.

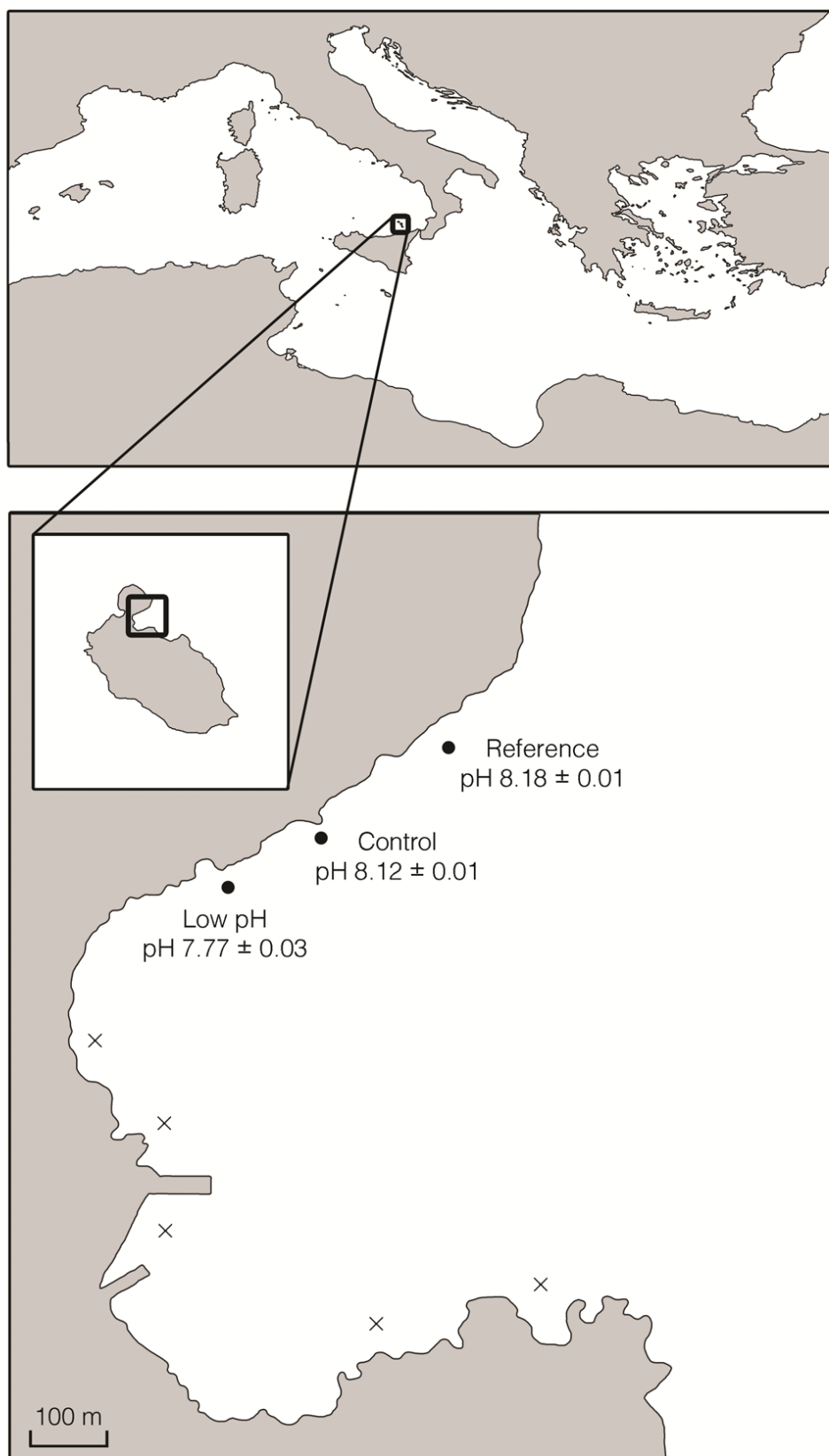


Figure 6.2: Map of the study area, Baia di Levante (Vulcano Island, Sicily), showing sampling sites 'Low pH' - pH  $7.77 \pm 0.03$ , 'Control' - pH  $8.12 \pm 0.01$ , and 'Reference' - pH  $8.18 \pm 0.01$ , with 'x' indicating the gas vents.

Table 6.1: Mean ( $\pm$  S.E.) seawater properties for the Low pH, Control and Reference site.  $\text{pH}_{\text{NBS}}$ , temperature, salinity, and total alkalinity ( $A_T$ ) are measured values. Seawater  $\text{pCO}_2$ , dissolved inorganic carbon ( $C_T$ ), bicarbonate ( $\text{HCO}_3^-$ ), carbonate ( $\text{CO}_3^{2-}$ ), carbon dioxide ( $\text{CO}_2$ ), saturation states for calcite ( $\Omega_{\text{calcite}}$ ) and aragonite ( $\Omega_{\text{aragonite}}$ ) are values calculated using the carbonate chemistry system analysis program CO2SYS

	Low pH	Control	Reference
$\text{pH}_{\text{NBS}}$	$7.770 \pm 0.026$	$8.117 \pm 0.012$	$8.184 \pm 0.011$
Temp ( $^{\circ}\text{C}$ )	$19.53 \pm 0.07$	$19.53 \pm 0.06$	$19.54 \pm 0.12$
Salinity (psu)	$38.18 \pm 0.01$	$38.19 \pm 0.01$	$38.14 \pm 0.02$
$A_T$ ( $\mu\text{mol kg}^{-1}$ )	$2594 \pm 26.19$	$2542 \pm 18.86$	$2533 \pm 7.98$
$\text{pCO}_2$ ( $\mu\text{atm}$ )	1311.3	517.2	428.3
$C_T$ ( $\mu\text{mol kg}^{-1}$ )	2491.4	2273.7	2225.4
$\text{HCO}_3^-$ ( $\mu\text{mol kg}^{-1}$ )	2347.7	2059	1988.58
$\text{CO}_3^{2-}$ ( $\mu\text{mol kg}^{-1}$ )	101.5	198	223
$\text{CO}_2$ ( $\mu\text{mol kg}^{-1}$ )	42.2	16.6	13.8
$\Omega_{\text{calcite}}$	2.368	4.619	5.203
$\Omega_{\text{aragonite}}$	1.543	3.008	3.389

### 6.2.3 Reciprocal transplant and oxygen uptake

Using an orthogonal design, six cages per treatment ( $10 \times 10 \times 10$  cm) were distributed across four treatments, each containing an individual *H. trunculus*. Individuals from the Low pH site were (i) transplanted into Control site, or (ii) re-transplanted into the Low pH site, and individuals collected from the Control site were (iii) transplanted into Low pH site, or (iv) re-transplanted into the Control site. After 14 days exposure, the cages were recovered and transported to a reservoir tank prior to placement into respirometers for oxygen uptake measurements within 30 min of collection.

Rates of oxygen uptake were used as a proxy for metabolic rate and measured using stop-flow respirometers (volume 460 ml). The respirometers were immersed in a reservoir tank ( $40 \times 60 \times 30$  cm) to maintain natural temperature ( $19.53 \pm 0.06$   $^{\circ}\text{C}$ ), and supplied with fully oxygenated filtered seawater from either the Control ( $\text{pH } 8.1 \pm 0.1$ ) or Low pH ( $\text{pH } 7.8 \pm 0.1$ ) site. Before determination of oxygen uptake, individuals of *H. trunculus* were placed into a respirometer, and left for 1 h to allow recovery from handling stress. At this point, a water sample was taken via syringe, and the circulation of seawater from the reservoir to the respirometers was stopped. This sample was passed over a  $\text{PO}_2$  electrode (E101/E5046 polarographic  $\text{O}_2$  electrode, Loligo systems, Denmark) connected to an oxygen meter (Oxygen Meter model 781, SI Strathkelvin Instruments Limited, Scotland) that was calibrated to 100% aerated seawater. A second water sample was taken from each respirometer after 1 h (maintained above 17kPa), and the rate of oxygen uptake was determined as the change in  $\text{PO}_2$  between the first and second water sample. This was multiplied by the solubility coefficient for oxygen, adjusted for salinity and temperature (Harvey, 1955), and the volume of water within each respirometer. Whole animal rates of oxygen uptake were calculated as  $\mu\text{l O}_2 \text{ h}^{-1}$  and corrected for standard conditions for temperature and pressure (STPD), and expressed as  $\mu\text{mol}$



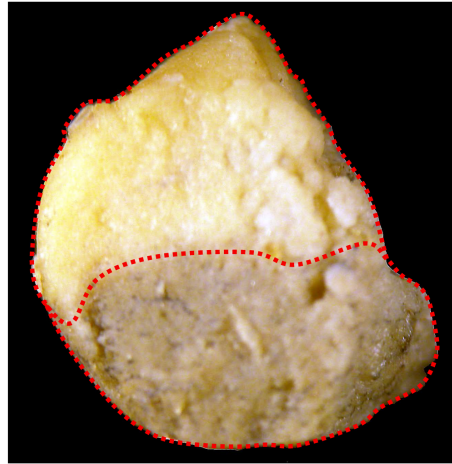


Figure 6.3: Digital image of *H. trunculus* visceral coil. Outlines (red dotted lines) traced on the images were used to estimate the gonadosomatic index (GSI). The gonad is the lighter colour positioned in the upper section.

$O_2 \text{ h}^{-1}$  STPD. Statistical differences between metabolic rates were analysed by ANOVA followed by Tukey's *post-hoc* test. All data sets were normally distributed and had equal variance.

#### 6.2.4 Sex determination, body size and gonads

*H. trunculus* is gonochoristic (Elhasni *et al.*, 2010), and hence, males were identified by the presence of a penis and lack of capsule gland, whereas females were identified by the presence of a vagina and capsule gland (Vasconcelos *et al.*, 2006). The gonads of *H. trunculus* are located in close proximity to the digestive gland (forming the visceral coil), and hence cannot be precisely dissected from the rest of the soma without damage and loss of gonadal tissue (Vasconcelos *et al.*, 2008). The gonads can, however, be distinguished from the digestive gland by differences in colouration, and therefore a visual gonadosomatic index (GSI) outlined by Vasconcelos *et al.* [2008] was used.

Measuring the GSI involves making a transverse cut in the medial zone of the visceral coil (digestive gland and gonad complex, immediately behind the gastric caecum). A 2-3mm thick section was cut, and a digital image taken by a camera (Nikon E4500) mounted on a dissecting microscope (10 × magnification; see Figure 6.3 for an example image). Outlines of the digestive gland and overlying gonad were traced using ImageJ (Abràmoff *et al.*, 2004) with GSI estimated as the relative proportion of gonad cross-sectional area in the total cross-sectional area of the digestive gland and gonad complex (see Figure 6.3).

Body size was determined by dissecting the body tissue from the shell, and measured as dry mass (g, 60 °C, 48 hours). Gonad mass was estimated using the estimated GSI (%) and measured body mass ( $\pm 0.1\text{mg}$ ; Ohaus Pioneer PA214C, Ohaus Corporation, Parsippany, New Jersey, USA). Shell thickness (outer lip) was measured three times for each individual in the growing tip along the anterior portion of the shell (dissection microscope, M5A, Wild Heerbruug, Switzerland) using the same individuals as for sex, and body and

gonad size. Differences in sex ratios were determined using Chi-Squared Test, using an estimated 50:50 ratio (based on Vasconcelos *et al.*, 2004). Statistical differences in body size and gonad size were determined using a two-way ANOVA of site (Low pH, Control, Reference) and sex (male, female). Shell length and thickness was tested with a one-way ANOVA of site. All data sets were normally distributed and had equal variance.

#### 6.2.5 Shell shape

##### Landmark configuration

Landmark based geometric morphometrics (GMM) provides the ability to capture the form of a structure (Rohlf & Marcus, 1993; Klingenberg *et al.*, 2002). This structure is designated through the choice of landmarks; homologous locations of the anatomical structure that can be accurately positioned in all individuals (Zelditch *et al.*, 2012). The 20 chosen landmarks and 19 sets of sliding semi-landmarks are displayed in Figure 6.4 with their definition outlined in Table 6.2. Landmarks were obtained by taking a digital image (Nikon PowerShot A2300 HD) of the ventral side of each individual, and then digitised using programs from the TPS suite (Rohlf, 2010). The method of sliding semi-landmarks allows a series of points to be designated along the outline of a shape between two landmarks. The specific position of these points is relatively arbitrary, as it is information about the curvature (or outline) as a whole that is of interest. These outlines can then be combined with landmark data in one analysis, providing a richer description of shape (Adams *et al.*, 2004). Overall, this provides a set of Cartesian coordinates (x,y) of the landmarks for each individual. Each set of coordinates was then scaled using measured shell length (digital callipers,  $\pm 0.01$  mm; distance between landmark 1 and 10, Figure 6.4a). This provides the relative position of each landmark in relation to the overall Cartesian shape conformation, and allows all of the landmarks to be considered simultaneously in the analysis, thus permitting a more complex and detailed description of shape.

##### Procrustes superimposition

The principal component of variation between individuals is size, hence, in order to assess for differences in shape among sites requires accounting for this variance. Therefore, the first step in GMM requires the use of a generalised Procrustes analysis (GPA, Rohlf & Slice, 1990) on all of the digitised landmark conformations. This procedure rotates all the landmark conformations into a common orientation, the conformations are then translated and superimposed using a least-square algorithm, and finally, size is standardised among conformations. Overall, GPA reduces the variability of the dataset, retaining only information on shape. Due to the procedure, four degrees of freedom are lost: one for minimising rotational differences, one for size standardisation, another two for translating configurations on the X and Y coordinate axes to superimpose their centroids. The superimposition is necessary because Procrustes shape space is curved, and traditional statistical analysis requires data to be in flat Euclidean space. The superimposition involves projecting the shape coordinates into a Euclidean space tangent to the Procrustes shape space.

##### Shape analysis

Shape description and intra-specific comparison between sites (Low pH, Control and Ref-

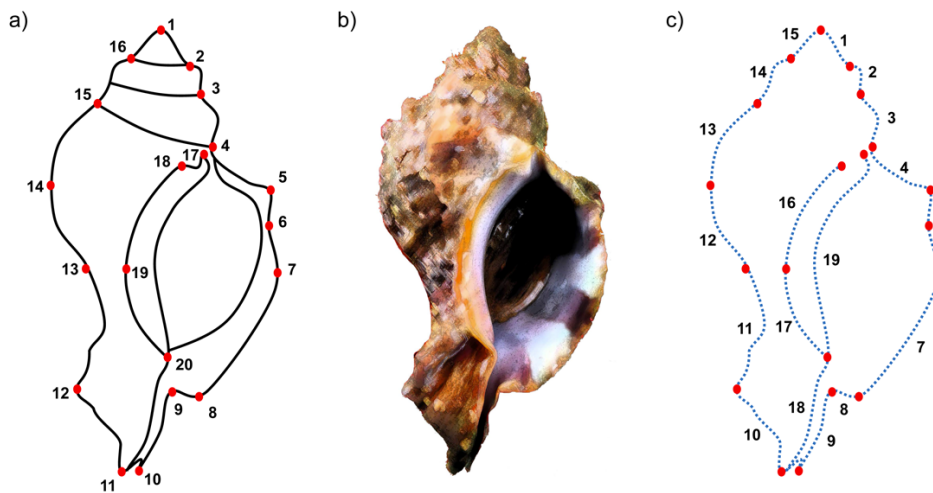


Figure 6.4: a) 20 Landmarks used for *H. trunculus*. Details of each landmark are outlined in Table 6.2; b) illustration of an example shell to aid interpretation of landmark placement; c) 19 Semi-landmark (outline) positions, each sliding semi-landmark represents the (numbered) dotted lines between the red landmarks designated in section a) and Table 6.2.

erence site), non-parametric MANOVA, canonical variate analysis (CVA) and thin-plate spline analysis were performed on the shape variables following GPA, for which MorphoJ (Klingenberg, 2011), PAST (Hammer *et al.*, 2001), and the R package 'Shapes' (Dryden, 2014) were used.

A complete assessment of the intra-specific differences was carried out using a non-parametric MANOVA (with 10,000 permutations,  $p$ -values Bonferroni corrected) on the Euclidean distances, with pair-wise distances tested between the three sites. CVA provides an ordination that maximises the separation of the group means relative to the variation within the sites (Klingenberg, 2011). To test the reliability of the discrimination between groups, the CVA results were tested by (bootstrapped) random forest discrimination (with an external cross validation with a held out dataset), which provides an aggregated ( $n = 100$ ) AUC value ( $\pm 95\%$  CI), in order to indicate the accuracy and margin of the discrimination between the three sites based on their shape. Using the held out dataset avoids the circular reasoning where a specimen is classified using methods that were calculated on samples that included that same specimen (Viscosi & Cardini, 2011).

The last approach for testing intra-specific differences between the three sites was the thin plate spline (TPS) algorithm (Bookstein, 1991), which takes its inspiration from D'Arcy Thompson [1917]. Thompson's approach described shape changes by superimposing a rectangular grid onto a (reference) starting shape and used mathematical equations to warp this grid into the shape of a different (target) shape. Similarly a "...way to think about ... [the] TPS ... is as if one form were printed on a transparent stiff plastic sheet [together with a set of square grids] and then manipulated by bending so that its 'shadow' takes on the prescribed landmark positions of the second form" (Zelditch *et al.*, 1992; Viscosi & Cardini, 2011). The TPS approach uses an interpolating function to produce smooth deformations (least bending energy) from the reference to target landmark configuration. Hence, specific shape features can be described in terms of the deformation grids.



Table 6.2: Landmark definitions for the twenty landmarks used in the geometric analysis. The terms ‘anterior (landmark 1)’ and ‘posterior (landmark 10)’ are used with reference to Figure 6.4.

Landmark	Description
1	Apex
2	First suture from the apex on the right-hand side
3	Second suture from the apex on the right-hand side
4	Final suture on the right-hand side adjoining the aperture
5	Tip of the spine extending from the posterior edge of the peristome
6	Base of the spine extending from the posterior edge of the peristome
7	Right-hand most aspect of the peristome
8	Anterior edge of the peristome
9	Joining of the peristome with the siphonal canal
10	Right-hand side of the tip of the siphonal canal
11	Left-hand side of the tip of the siphonal canal
12	Tip of the spine extending from the anterior body whorl and siphonal canal
13	Anterior edge of the body whorl
14	Left-hand most edge of body whorl
15	Suture on the posterior edge of the body whorl
16	First suture from the apex on the left-hand side
17	Posterior canal
18	Posterior edge of the inner lip
19	Left-hand most side of the inner lip
20	Base of the inner lip adjoining the siphonal canal

### 6.2.6 Population genetics

#### Total genomic DNA extraction

Tissue samples were collected non-destructively by taking a small portion of foot tissue, and preserved in 99% ethanol until being extracted. Following [Gonzalez-Tizon \*et al.\* \[2008\]](#), care was taken to ensure that the tissue sample was not contaminated by avoiding cutting the muscle tissue in close proximity to the proboscis (containing digestive enzymes) or collecting the sample from the external surface of the foot (with dark pigmentation). Following, the tissue collection, individuals were returned to their respective sites.

Total genomic DNA was extracted as in [Winnepenninckx \*et al.\* \[1993\]](#) from 20 mg of ethanol-preserved (99% ethanol) muscle tissue. The tissue was homogenised and incubated (overnight at 37 °C) in proteinase-K and CTAB buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0). The DNA was purified with phenol/chloroform/isoamyl alcohol (25:24:1), and then purified further using chloroform/isoamyl alcohol (24:1) and precipitated using 99% ethanol, overnight at -20 °C. After DNA centrifugation, the pellet was washed with 70% ethanol and air-dried. Finally, the DNA was dissolved in distilled water.

#### Microsatellite development and characterisation

The construction of the *H. trunculus* microsatellite-enriched genomic library used high quality DNA from five individuals which was pooled and enriched according to [Glenn & Schable \[2005\]](#). Preliminary testing of all loci was performed on DNA extracted from eight individuals with products electrophoresed on 2% agarose gels. Cloning, screening of the library for the presence of microsatellite arrays, DNA sequencing and PCR primer design were performed following [McKeown & Shaw \[2008\]](#). These methodologies can be described as follows:

The genomic DNA from the tissue sample was digested using *RsaI* restriction enzyme (New England Biolabs), ligated to matching linkers and amplified by polymerase chain reaction (PCR) using the linkers as PCR primers. Enrichment was performed by selective hybridisation of biotin-labelled repeat motif oligonucleotide probes (TG)<sub>12</sub>, (GA)<sub>12</sub>, (AAAT)<sub>8</sub>, (AACT)<sub>8</sub>, (AAGT)<sub>8</sub>, (ACAT)<sub>8</sub> and (AGAT)<sub>8</sub> to the PCR products. Hybridised complexes were captured using streptavidin-coated magnetic beads (DYNAL). Microsatellite enriched eluates were PCR amplified and cloned using the TOPO-TA cloning kit (Invitrogen). Recombinant colonies were identified by inactivation of the B-galactosidase gene, individually transferred into 50 µl of 10 mM Tris-HCL (pH 8.5) and incubated at 95 °C for 10 min to promote plasmid DNA release. One µl of each plasmid extract was submitted to PCR involving M13 forward and reverse primers.

A high proportion (80%) of sequences obtained from the *H. trunculus* library contained microsatellite arrays. However, only 13 loci displayed clear PCR amplicons when visualised on agarose, of which only 4 produced unambiguous genotypes upon population screening (GenBank accession numbers KJ765703-KJ765706). In all cases the loci that could not be reliably genotyped displayed multiple peaks that were not reduced by primer and/or PCR redesign. Loci producing clear PCR amplicons were then characterised by

genotyping the entire population sample. For population genotyping, an M13 tail (5'-TGTAACGACGGCCAGT-3') was added to the 5' end of each forward primer for flexible dye labelling.

For the four loci that could be unambiguously genotyped, each locus was individually amplified in a 10 µl reaction mixture containing ~100 ng of template DNA, 1X buffer, 2.0 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.1 pmol of forward primer, 0.2 pmol each of a fluorescent dye (Applied Biosystems) labelled M13 primer and the species specific reverse primer, and 0.2 µl of Taq DNA polymerase (Bioline, UK). PCR amplifications involved an initial denaturation step (95 °C for 3 min) followed by 25 cycles of 30 s at 95 °C, 30 s at the locus optimal annealing temperature ( $T_a$  - Table 6.3) and 30 s at 72 °C with a further 10 cycles then performed wherein the  $T_a$  was reduced to 50 °C. PCR products were size separated using an AB3500 DNA sequencer (Applied Biosystems) with allele inference performed using the GENEMAPPER software (version 4.1, Applied Biosystems). Allelic variation was detected at all locus, with allele sizes differing by expected multiples of their repeated motifs. Tests for linkage disequilibrium (LD) and deviations of genotype proportions from expectations of Hardy-Weinberg equilibrium were performed using default parameters in GENEPOP 4.0 (Rousset, 2008).

### Statistical analysis of microsatellite data

Genetic structure between the three sites was investigated using two different methods. Single- and multi-locus values estimating unbiased  $F_{ST}$ ,  $\theta$ , (Weir & Cockerham, 1984) were calculated in FSTAT, with the significance of pair-wise comparisons tested by 10,000 permutations of genotypes (Goudet, 1995). Secondly, genetic structure was investigated using a Bayesian clustering analysis in the program STRUCTURE (Pritchard *et al.*, 2000), which does not include the site location a priori, and instead attempts to identify clusters ( $K$ ) in a range of 1-4. Two models were used within STRUCTURE, a 'no admixture model' (as recommended for low  $F_{ST}$ ; Pritchard *et al.*, 2000) that assumes individuals are discretely from one population or another, and an 'admixture model' with correlated allele frequencies, which each individual draws some fraction of their genome from each of the  $K$  populations. Each model was run with a burn of  $10^6$  steps, followed by  $5 \times 10^6$  Markov Chain Monte Carlo (MCMC) runs.

### Mitochondrial genotyping

For mtDNA genotyping, a 602bp fragment of the cytochrome oxidase I gene was amplified using species-specific primers HtrucF (5'-ATATGGTCAGGGCTTGTGG-3') and HtrucR (5'-CCTGCAGGATCAAAGAACG-3') which were designed from the GenBank sequences (EU391577, Claremont *et al.*, 2008). PCR thermoprofiles were the same as for microsatellite loci. Purified amplicons were sequenced with the HtrucR primer using BigDye terminator kit (Applied Biosystems) on an ABI3500 sequencer. Sequences were edited, aligned and trimmed to a standard length (532bp) in BIOEDIT (Hall, 1999).

### Statistical analysis mitochondrial data

Genetic variation was described using indices of haplotype and nucleotide diversity ( $h$ , and  $\pi$ ; Nei, 1987) and their variances, calculated in ARLEQUIN (Excoffier *et al.*, 2005).

Differentiation among samples was assessed by global and pairwise  $\theta_{ST}$  with significances tested using 1000 permutations.

### 6.2.7 Population density

The densities of *H. trunculus* were estimated from an underwater visual census, with density expressed as numbers of individuals encountered during a timed search (3 min,  $\sim 5 \text{ m}^2$ ,  $n = 15$ ) over suitable habitat (rocky, sandy-mud and mud substrata; Peharda & Morton, 2006), while maintaining similar sampling effort.

## 6.3 RESULTS

### 6.3.1 Oxygen uptake

Metabolic rate was significantly increased by exposure to low pH ( $F_{3,20} = 17.78$ ,  $p < 0.001$ ). The mean  $\text{MO}_2$  of individuals collected and caged at the Low pH site (Low pH-Low pH) were significantly higher compared to all other treatments (Figure 6.5). In contrast, individuals of *H. trunculus* collected from, and caged, in the Control site (Control-Control) showed a significantly lower mean  $\text{MO}_2$  compared to all other treatments (Figure 6.5). When *H. trunculus* individuals were transplanted from the Control site into low pH conditions (Control-Low pH) and vice-versa (Low pH-Control), metabolic rate did not significantly differ from each other ( $p > 0.05$ ), and showed a mean  $\text{MO}_2$  approximately halfway between the other two treatments (Figure 6.5).

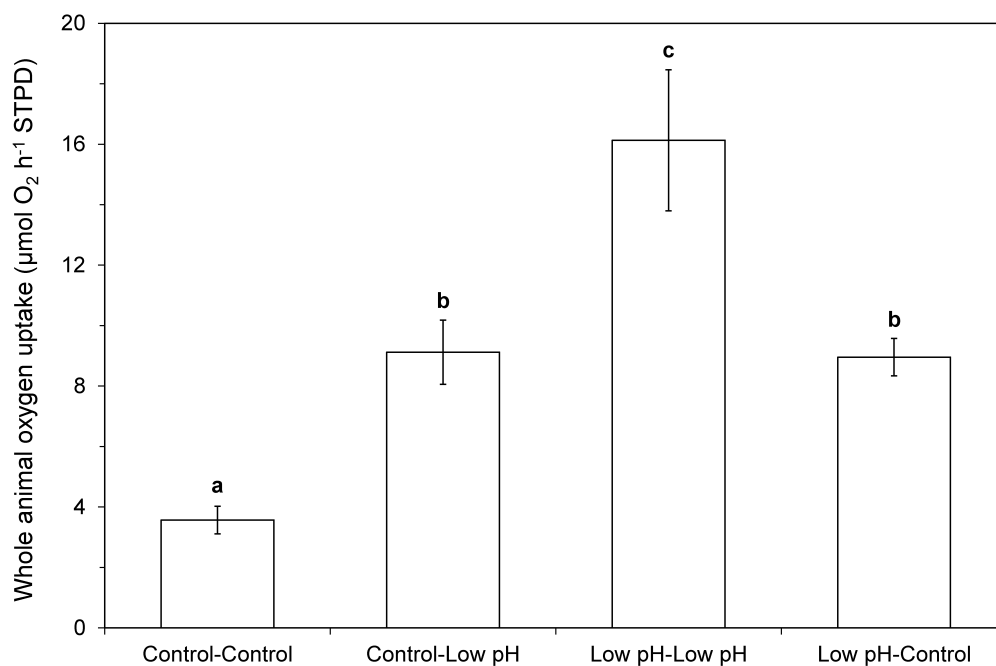


Figure 6.5: Mean oxygen uptake ( $\pm$  S.E.) of *H. trunculus* following a reciprocal transplant between the Low pH and Control site. Individuals were either (i) collected in the Control site and re-transplanted in the Control site (Control-Control), (ii) transplanted from the Control site to the Low pH site (Control-Low pH), (iii) re-transplanted within Low pH site (Low pH-Low pH) and (iv) transplanted from Low pH into the Control Site (Low pH-Control). Mean  $\text{MO}_2$  is expressed as  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ STPD}$ . Significantly different treatments are indicated by different lower case letters above the column (Tukey HSD,  $p \leq 0.05$ ).

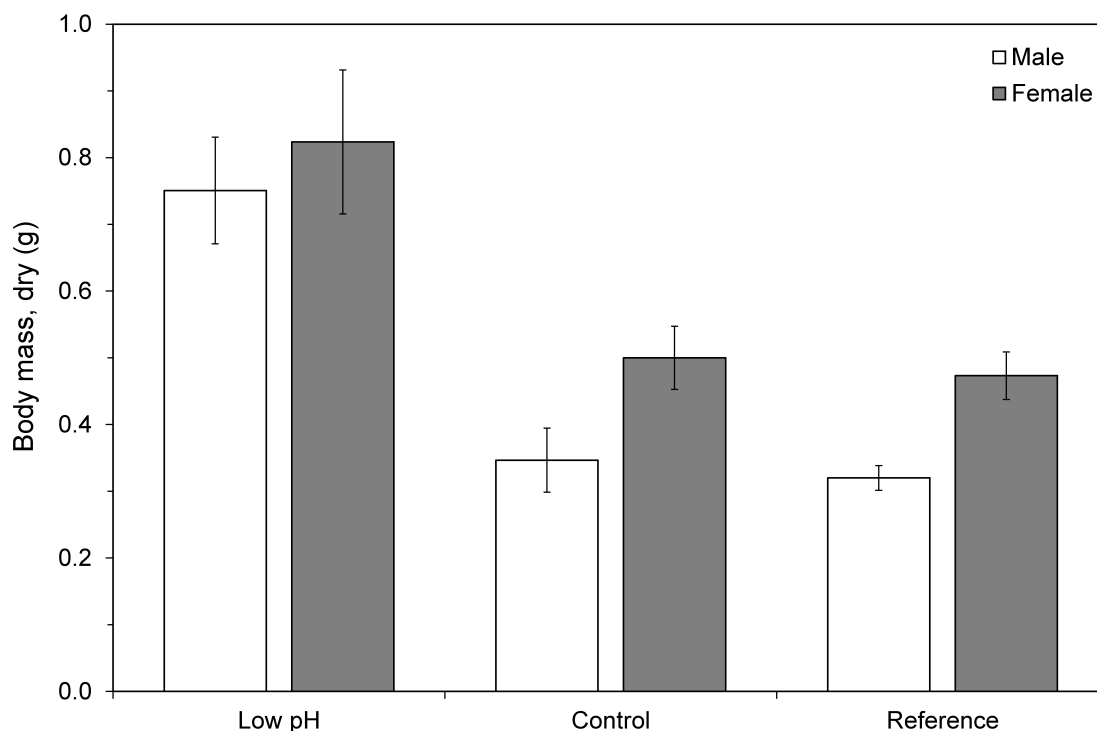


Figure 6.6: Mean dry body mass ( $\pm$  S.E.) of male (open bars) and female (grey bars) individuals (length  $44 \pm 1.2$  mm [SE]) collected from the Low pH, Control and Reference site.

### 6.3.2 Sex determination, body size and gonads

Significantly fewer females (compared to males) were present at the Low pH site (32.26%,  $\chi^2_{21,31} = 12.59$ ,  $p < 0.001$ ), while the sex ratio in the ambient pH sites did not significantly differ from an equal ratio (Control, 54.84% female; Reference, 45.83% female,  $p > 0.05$ ). Mean body size of individuals was significantly larger in the Low pH site ( $F_{2,66} = 22.37$ ,  $p < 0.001$ ; Figure 6.6), with female individuals larger than their male counterparts across sites ( $F_{1,66} = 7.30$ ,  $p < 0.01$ ; Figure 6.6). Similar to body size, gonad size was significantly greater in the Low pH site ( $F_{2,77} = 26.63$ ,  $p < 0.001$ , Figure 6.7a) compared to the ambient  $p\text{CO}_2$  sites. Gonad size was also reduced in females ( $F_{1,77} = 9.36$ ,  $p < 0.01$ , Figure 6.7b) compared to males.

### 6.3.3 Shell length and thickness

Mean shell length ( $F_{2,208} = 3.33$ ,  $p < 0.05$ ) and shell lip thickness ( $F_{2,66} = 4.36$ ,  $p < 0.05$ ) of individuals was significantly smaller in the Low pH site when compared to the ambient sites.

### 6.3.4 Shell shape

Shell shape significantly differed between the three sites (Mahalanobis distances, 1000 permutations, Low pH:Control site = 6.72; Low pH : Reference site = 8.5162; Reference : Control site = 5.9741; Bonferroni-corrected  $p < 0.003$  for all three pair-wise comparisons). The CVA demonstrated a similar clear ordination between the three sites (Figure 6.8). The first canonical variate (accounting for 71.59 % of the variance) clearly separates the

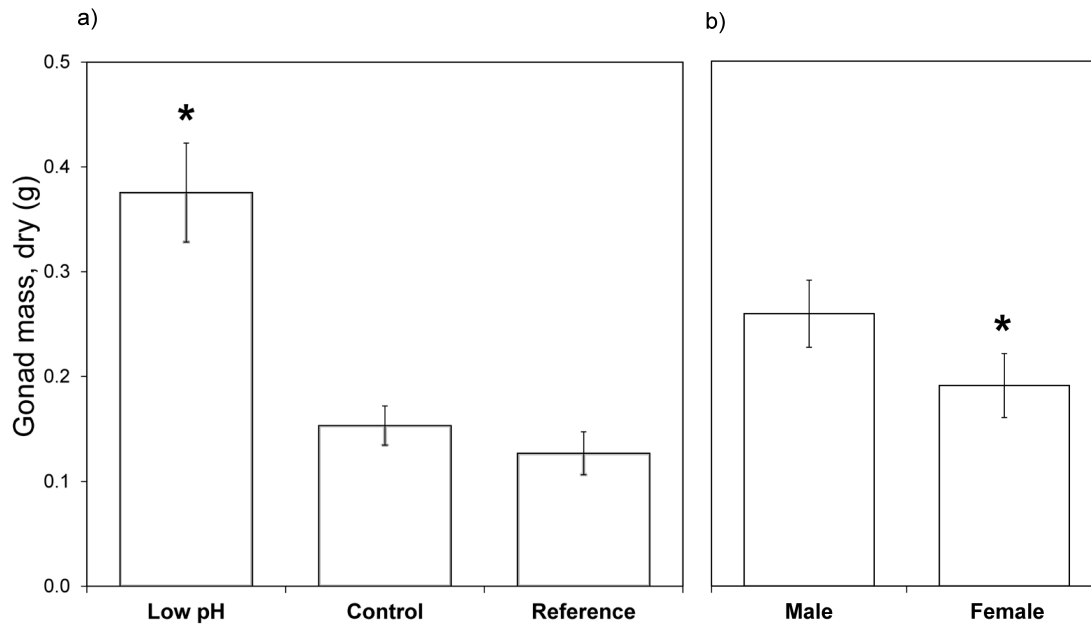


Figure 6.7: Mean gonad body mass ( $\pm$  S.E.) between a) the Low pH, Control and Reference site, and b) male and female individuals. Based on individuals of length  $44 \pm 1.2$  mm SE. Significantly different treatments are indicated by an asterisk.

Low pH site from both the Control and Reference site, with the Control and Reference site partially overlapping. The second canonical variate (accounting for 28.41 % of the variance) separates the Control site from the Low pH and Reference site (Figure 6.8). Accuracy of discrimination between the three sites was 58.74 % for the Low pH site, 72.52 % for the Control site and 72.91 % for the Reference site, resulting in a total reliability of 68.37 ( $\pm$  0.52)% of the held out test set, and overall discrimination accuracy of 67.49 ( $\pm$  0.75) %. AUC values were 0.76 ( $\pm$  0.005) for held out test set and 0.76 ( $\pm$  0.01) for the overall test set, indicating a fair accuracy (sensu Metz, 1978).

The TPS transformation grids show the change in mean shape (or consensus of landmark configuration). The mean shape does not considerably change between the Control and Reference site (Figure 6.9), however, the individuals from the Low pH site show clear changes in shell shape compared to both the Control site (Figure 6.10) and the Reference site (Figure 6.11). The qualitative differences between the Low pH site and the ambient pH sites, suggest a reduction in the overall thickness of the individual in the Low pH site. This was exhibited by (i) a reduction in the steepness of the apex (including apex truncation), (ii) less rugosity between the sutures of the whorls, (iii) the presence of a spine from the anterior edge of the peristome (absent in the Reference site), (iv) a reduced size of inner lip, (v) a reduction in the size of the shell adjacent to the siphonal canal (posterior to the body whorl), and finally (vi) an increased width between the edges of the siphonal canal.

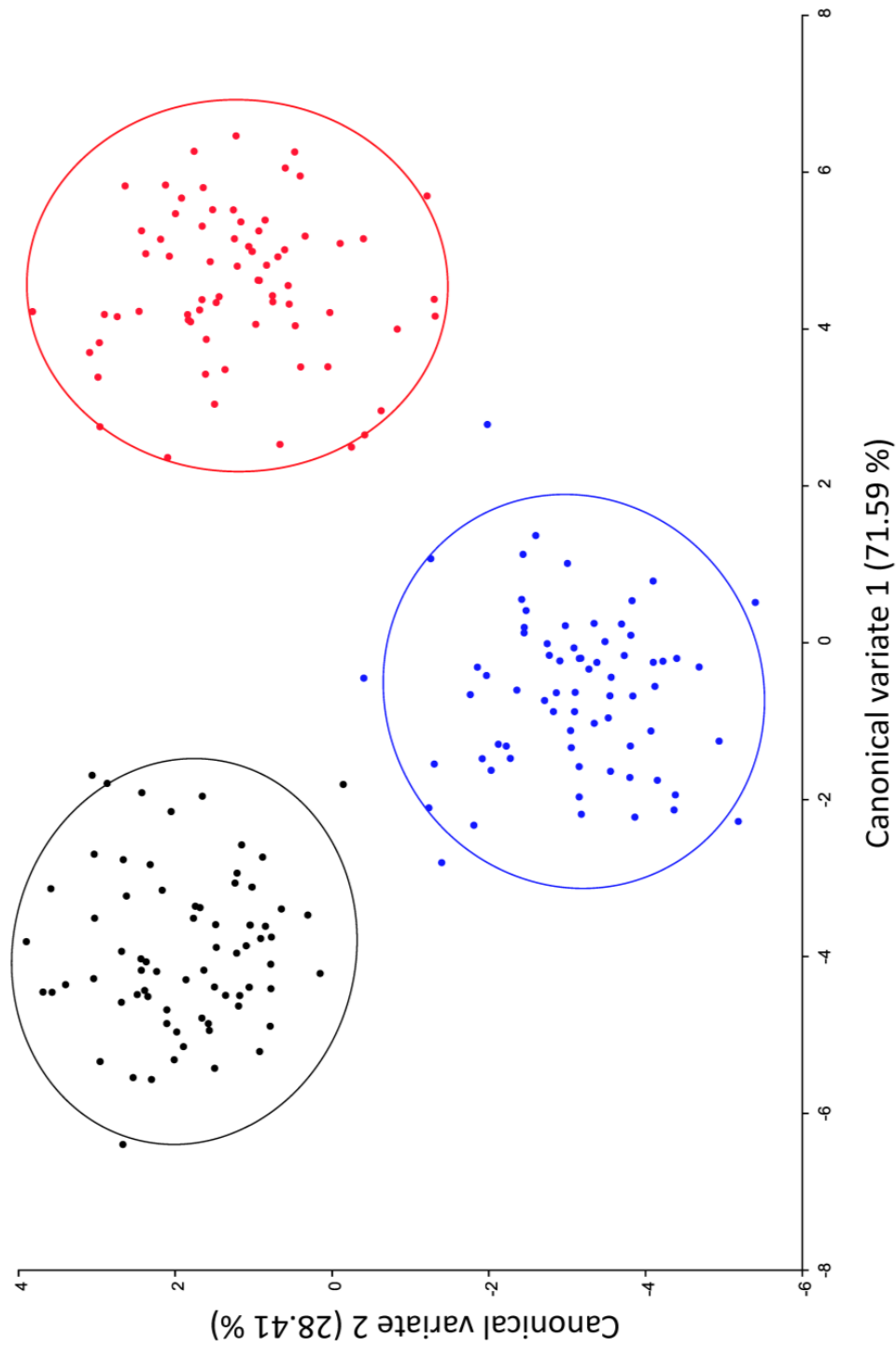


Figure 6.8: Canonical variate analysis of shell shape for discrimination between the Low pH (solid red), Control site (solid blue) and Reference site (solid black).

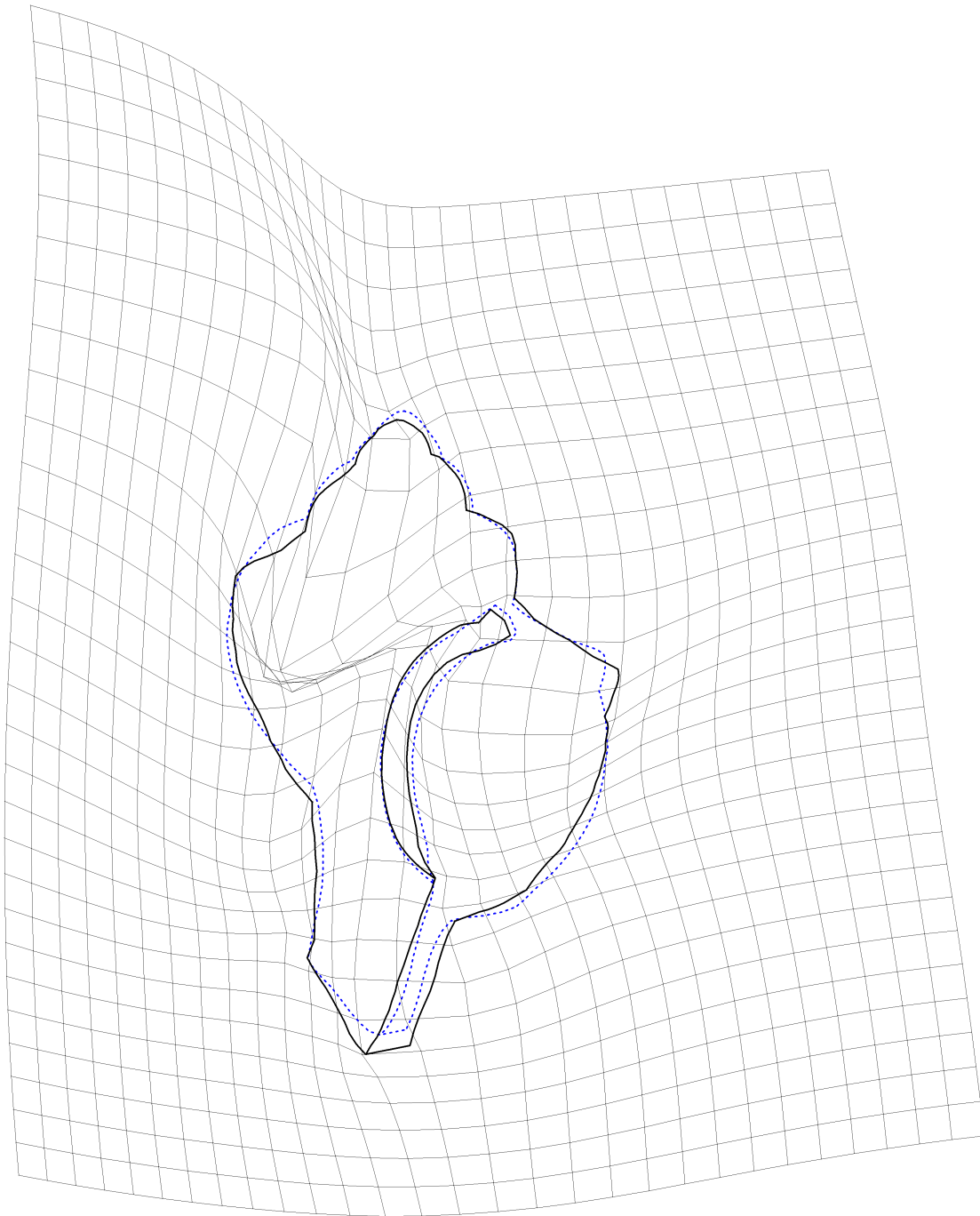


Figure 6.9: TPS transformation grid comparing the mean shell shape between the Reference site (dashed blue outline) and the Control site (solid black outline).



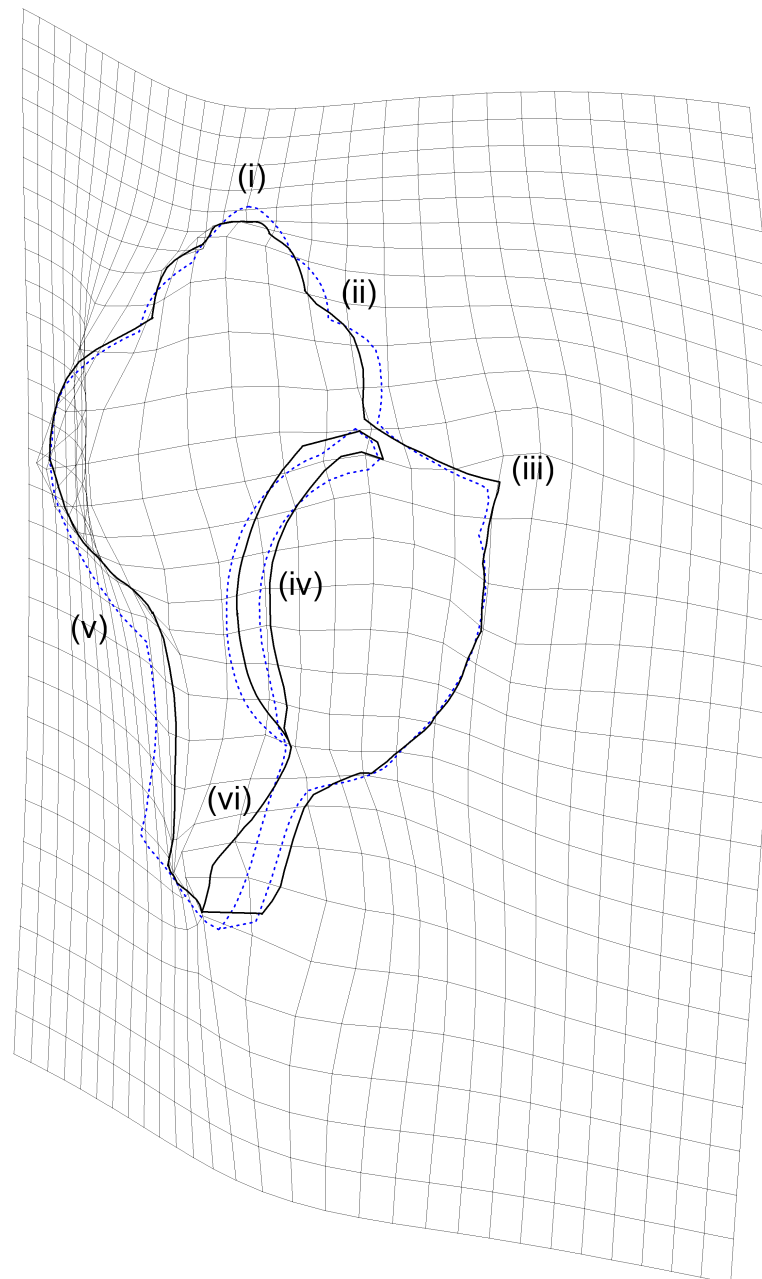


Figure 6.10: TPS transformation grid comparing the mean shell shape between the Control site (dashed blue outline) and the Low pH site (solid black outline). Six areas of interest are highlighted (i - vi) that demonstrate population differences in morphology, with further details in text (Section 6.3.4).

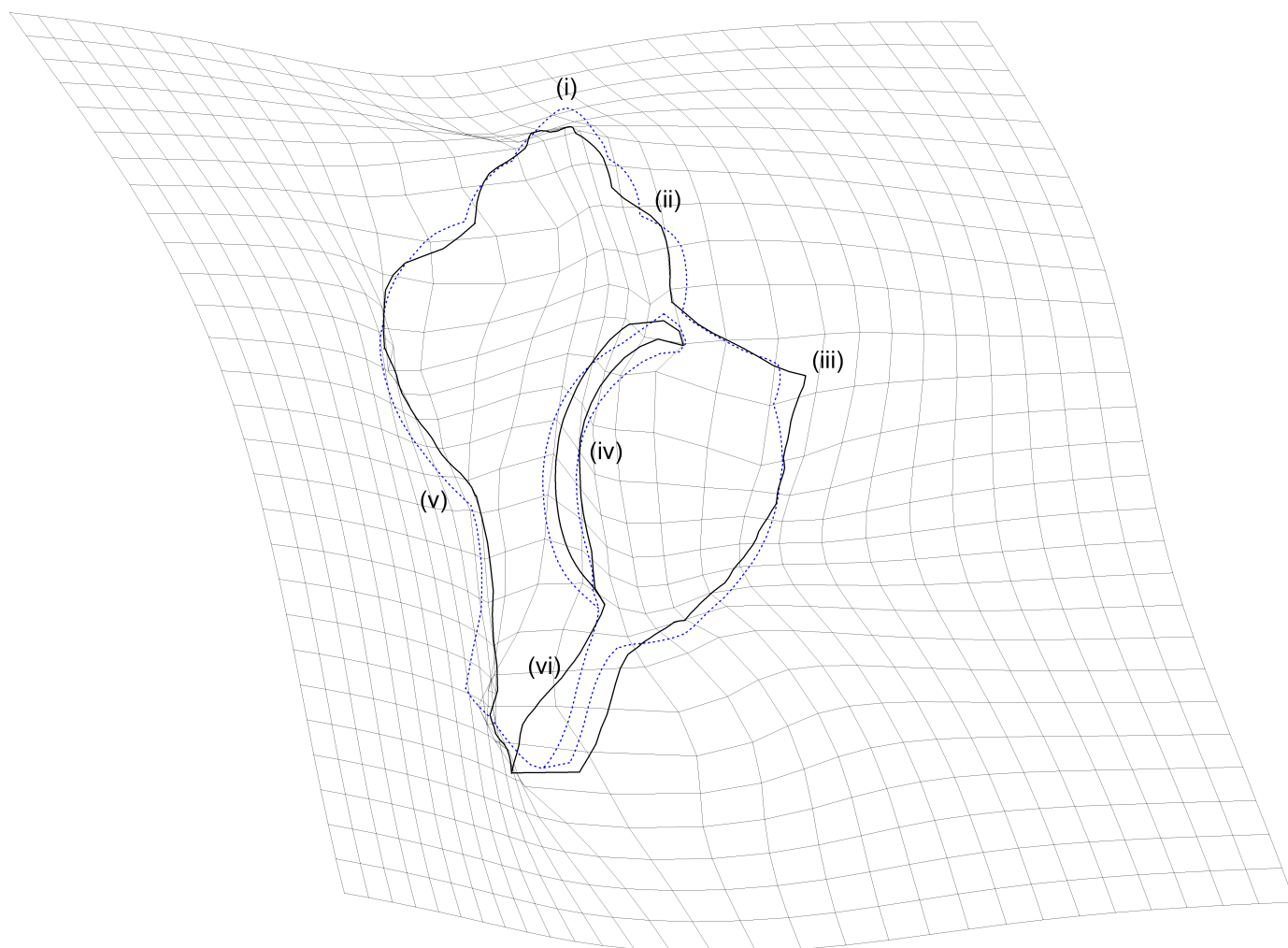


Figure 6.11: TPS transformation grid comparing the mean shell shape between the Reference site (dashed blue outline) and the Low pH site (solid black outline). Six areas of interest are highlighted (i - vi) that demonstrate population differences in morphology, with further details in text (Section [6.3.4](#)).

### 6.3.5 Population genetics

#### Microsatellite markers

Microsatellite markers are notoriously difficult to develop for molluscs with increasing evidence that this is due to genomic complexities within microsatellite flanking regions (McInerney *et al.*, 2010). Such complexities can include indels and mutations leading to PCR failure and/or repetitive DNA elements that lead to difficulties amplifying a single locus. The detection of multiple products at a number of the loci developed for *H. trunculus* suggests the presence of such cryptic repetitive DNA elements. With the exception of Htruc-2, which exhibited a significant heterozygote deficit, the loci reported here displayed no evidence of technical problems such as heterozygote deficits, null alleles, or variation in allele sizes not equal to repeat motifs, common among mollusc microsatellites (Panova *et al.*, 2008).

Table 6.3 shows the primer sequences and characteristics of the four (optimised) microsatellite loci. A significant linkage-disequilibrium was detected between Htruc-3 and Htruc-4 ( $p = 0.001$ ) and a significant heterozygote deficit was detected at Htruc-2 (Table 6.3). After population screening all individuals, no reduction in genetic variation was observed between sites (allele number,  $F_{1,20} = 0.21$ ,  $p = 0.66$ ; allele richness,  $F_{1,20} = 0.16$ ,  $p = 0.69$ ), or the two temporal samples (allele number,  $F_{1,20} = 0.24$ ,  $p = 0.63$ ; allele richness,  $F_{1,20} = 0.27$ ,  $p = 0.61$ ), and no significant interaction between the sites and temporal samples (allele number,  $F_{1,20} = 0.02$ ,  $p = 0.88$ ; allele richness,  $F_{1,20} = 0.03$ ,  $p = 0.87$ ).

Pairwise tests of differentiation in allele frequencies between sites (across all loci) found no significant differences between the Low pH, Control and Reference site for either of the temporal samples (Table 6.4 panel a & b). In the first temporal sample, the Reference site did differ to both the Low pH and Control sites at  $p < 0.05$ , however, the pooled sample (i.e. the two temporal samples combined) found no significant difference between sites (Table 6.4c), even at  $p < 0.05$ . This was supported by clustering analysis, which also reported no evidence for more than one genetic cluster (all  $p < 0.05$ ). Therefore, this indicates that there is no significant breakdown in gene flow between sites.

The most striking feature of the microsatellite data is the highly significant (multi-locus)  $F_{IS}$  values for the Low pH site, indicating a deficit of heterozygotes compared to the expectations of a randomly mating population (Figure 6.12). Pair-wise differentiations of variation between the sites also support this finding with statistically significant differences between the Low pH site and the Control site ( $p = 0.028$ ), the Low pH site and the Reference site ( $p = 0.035$ ), but no significant difference between the Control and Reference site ( $p = 0.555$ ). Mean relatedness between individuals was significantly reduced in the Low pH site, with the Control and Reference site showing similar levels of relatedness (Figure 6.13).

Table 6.3: Primer sequences and characteristics of 4 microsatellite loci optimised for *H. trunculus*, including repeat motif observed in the clones used to develop those loci obtained from the *H. trunculus* library and optimal annealing temperature ( $T_a$ ) for each primer pair. Allele numbers ( $N_a$ ) and size range (including M13 forward primer tail) in base pairs (bp), observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity and  $p$ -values for tests of fit to Hardy-Weinberg equilibrium genotype proportions (pHW).  $p$ -values in bold denotes values  $< 0.05$ .

Locus	Primer Sequences (5'-3')	Repeat motif in cloned allele	Ta (°C)	N <sub>a</sub>	Size range (bp)	H <sub>O</sub>	H <sub>E</sub>	pH <sub>W</sub>
<i>Htruc-1</i>	F: AAAAATGCAGAGGAGGCTGA	(AC) <sub>7</sub>	58	4	265-271	0.667	0.664	0.9919
	R: GAAGGCGTTGTAAGTTGTTGC							
<i>Htruc-2</i>	F: GTCGGGAATCCTCCACTGTA	(GAAT) <sub>4</sub> (TGAT) <sub>5</sub>	58	3	188-204	0.362	0.533	<b>0.0029</b>
	R: TGGCAGATATGTAAGTTGGTTAATG							
<i>Htruc-3</i>	F: CTGTTTCCGCCCTCTAAAC	(AC) <sub>15</sub>	58	6	98-108	0.769	0.801	0.5998
	R: AAAAAGCTTATTTTGGTGCAG							
<i>Htruc-4</i>	F: CCGTCTTCTGTCATGTGGAG	(TG) <sub>15</sub>	58	7	109-121	0.771	0.729	0.8335
	R: AAATTCAATCATGCAAAACCTG							

Table 6.4: Pairwise genetic differentiation (across loci) between sites (Low pH, Control and Reference) for a) temporal sample 1, b) temporal sample 2, and c) temporal samples 1 and 2 pooled. The lower triangular matrix of each square reports the unbiased  $F_{ST}$  estimator [Weir & Cockerham, 1984](#), and the upper triangular matrix of each square reports  $p$ -value of the respective exact test of allele frequency homogeneity.

a) Temporal sample 1				b) Temporal sample 2			
Low pH	Low pH	Control	Reference	Low pH	Control	Reference	
		$p = 0.88$	$p = 0.033$		$p = 0.28$	$p = 0.43$	
Control	-0.0026		$p = 0.033$	Control	0.0086	$p = 0.33$	
Reference	0.0194	0.0165		Reference	0.0023	0	
c) Samples pooled							
Low pH	Low pH	Control	Reference	Low pH	Control	Reference	
		$p = 0.08$	$p = 0.067$			$p = 0.067$	
Control		0.0069		Control		$p = 0.067$	
Reference	0.0093	0.0074		Reference	0.0093	0.0074	

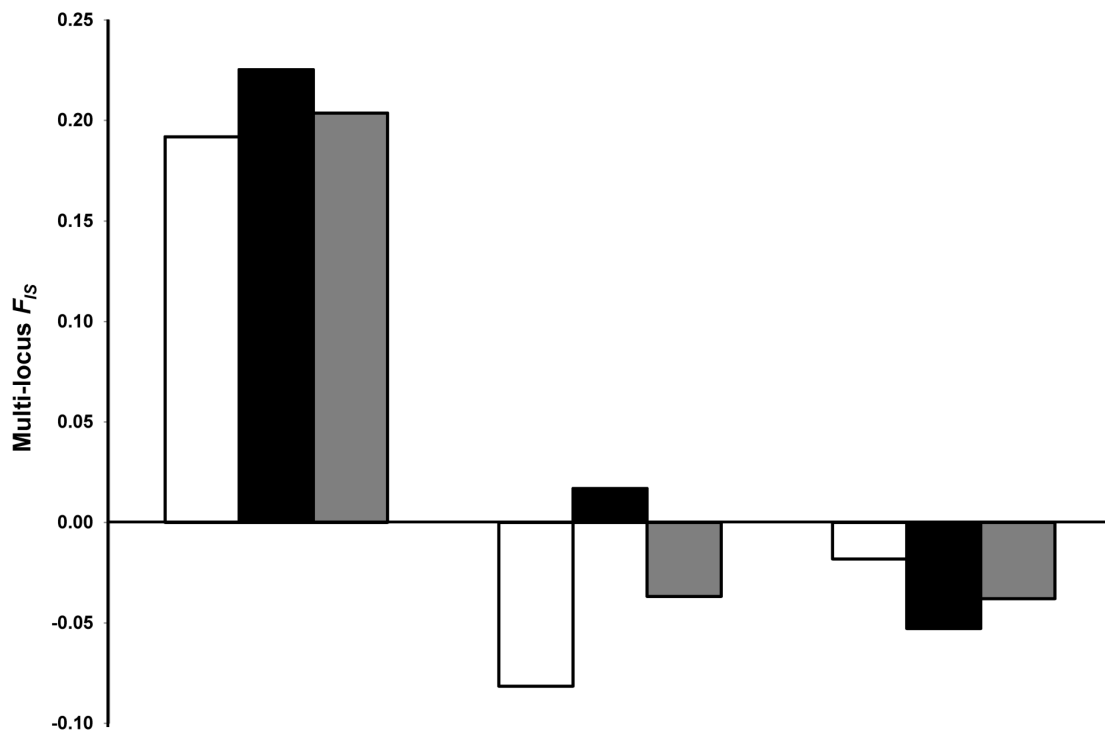


Figure 6.12: Multi-locus  $F_{IS}$  values for the Low pH, Control and Reference site. Temporal sample 1 - solid white, temporal sample 2 - solid black, pooled temporal samples - solid grey.

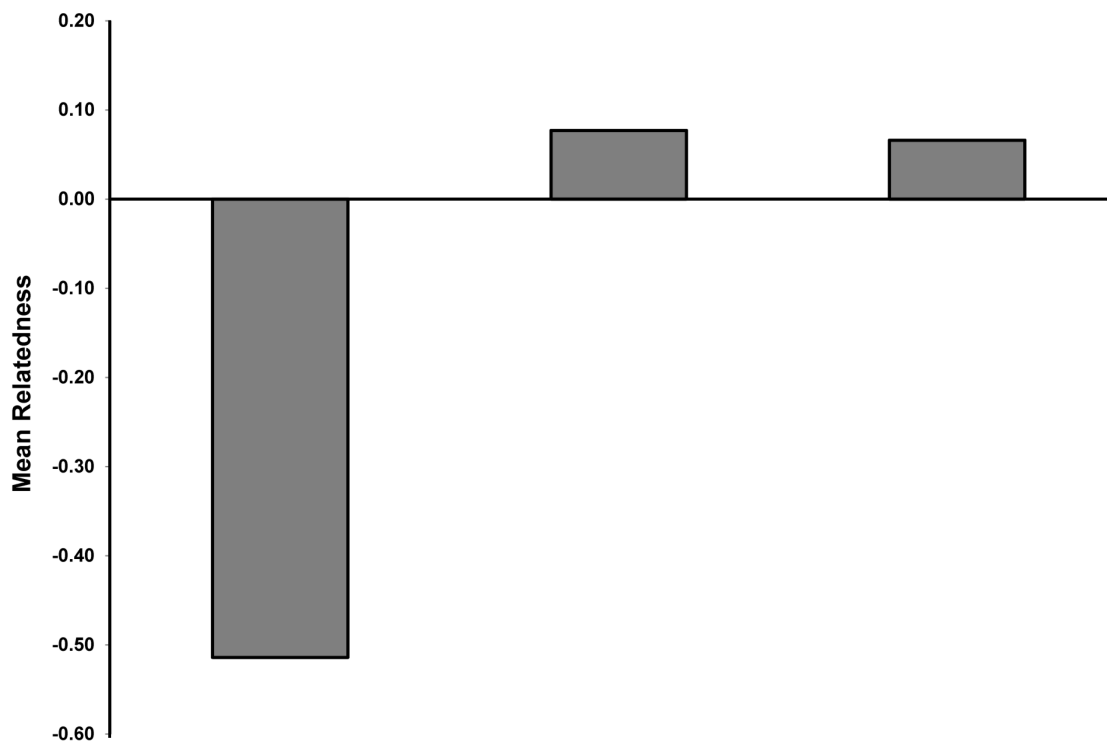


Figure 6.13: Mean relatedness for individuals in the Low pH, Control and Reference site

### Mitochondrial Markers

Overall haplotype diversity was  $0.405 \pm 0.091$  and nucleotide diversity was  $0.0072 \pm 0.004$ , with similar levels of variability observed across the three sites (Table 6.5). Pairwise tests of  $\theta_{ST}$  demonstrated no significant differences between the sites (Low pH:Control =  $-0.0126$ ,  $p = 0.80$ , Low pH:Reference =  $-0.0047$ ,  $p = 0.39$ , Control:Reference =  $-0.0100$ ,  $p = 0.58$ ) Therefore, the mtDNA homogeneity among sites is concordant with the nuclear (microsatellite) pattern, supporting the observation that there is no significant breakdown in gene flow between sites.

Table 6.5: Haplotype ( $h$ ) and nucleotide ( $\pi$ ) diversities and associated standard deviations for the three sites, and their mean.

	Low pH	Control	Reference	Mean
<b><math>h</math> (SD)</b>	0.4636 (0.068)	0.4528 (0.061)	0.3001 (0.064)	0.405 (0.091)
<b><math>\pi</math> (SD)</b>	0.0074 (0.0042)	0.0080 (0.0044)	0.0063 (0.0036)	0.0072 (0.004)

#### 6.3.6 Population density

The population density was significantly reduced in the Low pH site compared to the ambient pH sites ( $F_{2,42} = 41.34$ ,  $p < 0.001$ ). Across the three sites, the density of *H. trunculus* fell from  $36 \pm 6.03$  individuals per  $10 \text{ m}^2$  in the Reference site, down to  $3.73 \pm 0.91$  individuals per  $10 \text{ m}^2$  in the Low pH site.

## 6.4 DISCUSSION

This study submits that ocean acidification increases the basal cost of living for *H. trunculus*, with individuals revealing an altered energy allocation strategy. This was demonstrated through elevated  $p\text{CO}_2$  conditions causing an increase in body size, and due to the increased body size, potentially increased gonad size. However, this also resulted in a simultaneous negative effect on the size, thickness and shape of the shell. When scaled up to the population level, it was observed that chronic elevated  $p\text{CO}_2$  conditions caused a reduction in population size and an altered sex ratio (decreased number of females). This suggests that there are key changes in demography that are likely to alter population dynamics. This study also observed genetic signatures of increased variance in reproductive success among individuals (despite high gene flow between the three sites). Collectively these results demonstrate that elevated  $p\text{CO}_2$  conditions can cause impacts beyond the individual and modify demographic and microevolutionary processes, that if translated into future high  $\text{CO}_2$  oceans are likely to alter eco-evolutionary trajectories.

#### 6.4.1 Metabolic rate

Exposure to low pH conditions increased the metabolic rate of *H. trunculus*, meaning that individuals exhibited increased energetic demand under ocean acidification, likely due to disturbances of extra- and/or intra-cellular acid-base homeostasis (Stumpp *et al.*, 2011, 2012a; Calosi *et al.*, 2013a). Those individuals either entering or leaving the acidified area

demonstrated similar changes in metabolic rate, and the ability to adjust their physiology with some acclimatisation (via phenotypic plasticity). However, not to the same extent as 'native' individuals, whereby chronic exposure to low pH conditions provided some measure of non-reversible acclimatisation (Sunday *et al.*, 2014). Acclimatisation can buffer populations against the immediate impacts of ocean acidification and even provide time for adaptation to catch up (Chevin *et al.*, 2010). However, acclimatisation can also come at a cost, resulting in stress-induced energetic trade-offs.

#### 6.4.2 Energetic trade-offs

Environmental factors modulate metabolic activity through alterations in energy demand and/or availability. Should insufficient energy be available, then stress-induced trade-offs between energy demanding processes can occur (e.g. growth, shell maintenance and reproduction, Dahlhoff *et al.*, 2002). Here, a decoupling of growth and shell maintenance was observed in the Low pH site, demonstrated by an increased body size (almost double), but reduced shell size (reduced shell length and thickness). Moreover, the alterations in shell shape suggest a less robust shell structure (based on the geometric morphometrics), however, this couldn't be empirically tested (e.g. shell strength) due to the non-destructive approach. A similar opposing energy allocation strategy between growth and calcification was observed in larvae of the European lobster *Homarus gammarus* (L.) which developed less calcified exoskeletons and yet maintained their growth rates under high CO<sub>2</sub>/low pH conditions (Arnold *et al.*, 2009). The ability of organisms to regulate pH at the site of calcification is energetically costly (Palmer, 1983), and therefore increases in energy partitioning towards maintaining acid-base homeostasis may reduce net calcification rates (Stumpp *et al.*, 2012a). Reduced shell thickness may also lessen any growth-limiting effect that the shell structure has on somatic tissue growth (Palmer, 1983), allowing for a larger body mass (assuming sufficient energy is available). This combination would, however, likely increase the susceptibility of *H. trunculus* to predation in the acidified area. Regardless, in terms of fitness, any changes in the energetics (as suggested by the changes in growth and calcification) could influence the energy available for reproduction, and therefore directly affect the reproductive output of individuals.

When considering both the GSI and body size in combination, it was found that gonad mass was actually increased in the low pH site. Body mass has previously demonstrated to be an important attribute in determining fecundity in *H. trunculus* (e.g. Lahbib *et al.*, 2009), and therefore the observed increase in body size could result in a greater reproductive investment. This does make the assumption that climate-driven stressors do not prevent individuals from achieving the advanced stages of gonad development (e.g. Moore *et al.*, 2011), despite the gonads increasing in size. Positive effects of ocean acidification on reproduction are not common, although elevated pCO<sub>2</sub> has been previously demonstrated to have positive effect on the clutch size (and numbers of eggs per clutch) of marine fishes (Miller *et al.*, 2013). Increases in reproductive investment could be used to offset the negative impacts of environmental stressors on reproductive performance and subsequently increase offspring survival (Paul *et al.*, 1993; Miller *et al.*, 2013).

When scaling from the individual to the population, there is a need to consider whether any responses are sex-specific, since any disproportionate effects on either gender could subsequently affect population demographics. The energetic costs of reproduction are generally greater for females (Miller *et al.*, 2013), and when considering *H. trunculus*



specifically, the females may be required to store sperm obtained from copulation over long periods, with the spermatozooids being oriented and sustained by the female (Gharsallah *et al.*, 2010), potentially representing an energetically costly process (Lahbib *et al.*, 2009). This suggests a mechanism through which population-level consequences could occur. If the cost of living in elevated  $p\text{CO}_2$  conditions is greater for female *H. trunculus*, this could directly impact their survival (causing a skewed sex ratio) with the additional potential to influence their reproductive investment. Sex-specific responses to climate driven stressors have previously been observed in other gastropod species, with differences in foraging rates and growth, and ultimately fitness (e.g. Vaughn *et al.*, 2014).

#### 6.4.3 Population-level

Despite the absence of population differentiation between sites, the most important feature of the genetic data was the large highly significant  $F_{IS}$  values reported for the low pH site compared to non-significant values reported for both ambient pH sites. This suggests that for *H. trunculus*, some aspect of the acidified environment results in greater departures from Hardy-Weinberg equilibrium in the form of heterozygote deficits. Such site-specific heterozygote deficits could be generated by inbreeding, natural selection acting on the genetic markers, or spatial/temporal structure within samples known as the Wahlund effect (Wahlund, 1928). As the significant multi-locus  $F_{IS}$  values resulted from significant values at the majority of individual loci, locus-specific selection effects are unlikely. Inbreeding effects within a small population can also be discounted, as the low pH site sample reported significantly reduced mean individual relatedness values compared to the Control and Reference sites. For a Wahlund effect to be observed a change in allele frequencies must occur within the geographical scale of the sampling.

Due to the high gene flow throughout the studied region, such heterogeneity in allele frequencies is most likely generated by increased variances in reproductive success among individuals, rather than (a temporally stable) population substructuring *per se*. Such variances in reproductive success among individuals have been widely reported for many highly fecund marine invertebrates (Hedgecock & Pudovkin, 2011). Within such a system, this means that large numbers of individuals do not contribute to recruitment in a given cycle. This results in short term genetic drift, generating genetic differences among groups of recruits even though they are all derived from a single population. These differences among groups then serve to generate the observed heterozygote deficits and decreased mean relatedness within samples.

Variances in reproductive success may be driven by any biotic or abiotic factors influencing fitness, and ocean acidification has been shown to strongly influence recruitment processes (Byrne, 2011). Previous studies have also demonstrated that climate driven stressors may result in a reduction in the proportion of the population that are reproductively active and an increase in reproductive failure years (such as in the limpet *Patella vulgata*, Moore *et al.*, 2011). The significantly reduced proportion of females in the Low pH site would represent an obvious mechanism that could increase variance in reproductive success among males, however, altered energy allocations and/or other cryptic factors may also contribute.

The increased variance in reproductive success and consequent increased random genetic drift (within and between cohorts) will, in theory, hinder adaptive evolution in re-

sponse to ocean acidification. Acclimatisation can represent an important mechanism for maintaining population performance under stressful conditions (Sunday *et al.*, 2014). However, any energetic trade-offs may result in previously unforeseen population consequences, and unless adaptive evolution can 'catch up' and sufficiently improve population fitness (Lande, 2009), there could be serious implications for population dynamics and ecosystem functioning. This study demonstrates that within a highly (genetically) connected system elevated  $p\text{CO}_2$  concentrations are driving individual and population level changes that will impact eco-evolutionary trajectories and highlights the need for deeper understanding of the links between the individual and (often unknown, Harvey *et al.*, 2014) population demographics, in order to predict and manage the consequences of climate change.

## GENERAL DISCUSSION

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## 7.1 OVERVIEW

Ocean acidification and warming represent two of the greatest challenges of the 21<sup>st</sup> century (Borja, 2014), and understanding how they will influence whole ecosystems and their functionality is crucial for effective management. The impacts of ocean acidification and warming on natural systems remains poorly understood, with past studies tending to focus on individual responses of marine species to single climate change stressors (Dupont & Pörtner, 2013; Kelly & Hofmann, 2013). The aim of this thesis was to investigate how ocean acidification and global warming could affect species and community assemblages, and determine the consequences for population-, community-, and ecosystem-level processes.

The main findings of this thesis are outlined in the following sections, along with a discussion into the insights gained as a result of the findings presented in this thesis, and finally, this chapter will finish with recommendations for the direction of future research.

## 7.2 FROM INDIVIDUAL TO ECOSYSTEM

### 7.2.1 *Individual physiology vs. realism in climate change experiments*

Understanding the response of marine organisms to the environmental conditions predicted with future climate change requires an appreciation of both the direct (organismal physiology) and indirect effects (species interactions). The first step of moving beyond single species responses, however, is to understand which traits are likely to be affected, and ultimately assess whether particular life-history characteristics can describe the sensitivity (or resilience) of an organism to future climate change. In Chapter 2, a meta-analysis was presented that suggested there are ‘winners’ and ‘losers’ in climate change, alongside some predictable trait-based variation in sensitivity/resilience that can be attributed to taxonomic groups and/or life stages. Importantly, it was also demonstrated that the combination of ocean acidification and warming generally interacted synergistically. Although such synergetic interactions are relatively common in the natural environment (e.g. Sala *et al.*, 2000; Harley *et al.*, 2006), they are of particular concern because they are unpredictable, and will limit the capacity to predict and manage the potential future impacts from single-stressor studies alone.

Much of research to date has tended to focus on the performance and survival of individual species to single climate stressors, over relatively short durations. While such research is invaluable in determining some of the specific physiological or behavioural mechanisms that dictate an organism’s resilience (or sensitivity), they might be lacking in their realism for determining the structure and functioning of future ocean ecosystems in the face of climate change. Predictions for the ecosystem-level consequences of climate change will also need to consider relative shifts in fitness and performance, changes in the community structure and biodiversity, as well as alterations to a species’ distribution and trophic interactions. Subsequently, the experimental manipulations on individual species to single stressors will likely be unable to capture this complexity, and an imperative remains to place the performance of the individual into a context (e.g. lifetime fitness costs in response to long-term exposure, Gaylord *et al.*, 2015). Importantly, however, while studies investigating the mechanistic underpinnings of responses to climate change cannot be simply scaled up to ecosystem-level responses, it must be stressed

that those same mechanistic studies help explain how and why responses are being observed at higher levels of biological organisation. Therefore, the principal objective of future climate change research should be to scale up from the individual- to ecosystem-level effects, but using a variety of collaborative experimental approaches.

### 7.2.2 *Environmental and biological context*

The responses of marine organisms to both ocean acidification and global warming cannot be considered in absolute terms (i.e. +4 °C), because (for example), the effect of temperature will be highly dependent on where the organism currently resides in relation to their thermal optimum (Stillman, 2003; Gianguzza *et al.*, 2014). Increases in temperature may be beneficial to those organisms on the cold side of their thermal performance curve, however, that same increase in temperature may have a negative effect on those species already at, or beyond, their thermal optimum. Therefore, organisms, populations and ecological communities will not be responding to the global mean change in environmental conditions, rather they will be influenced by variability around the mean at local or regional scales (see Walther *et al.*, 2002; Burrows *et al.*, 2014) and so responses must be considered with a local context, such as which ambient (control) conditions the treatments are being compared to.

With ocean acidification in particular, calcifying organisms (compared to non-calcifying organisms) are considered to be more negatively impacted by the conditions predicted for the future (Chapter 2), or to be more specific, those organisms with a reduced ability to maintain their acid-base homeostasis (e.g. crustaceans vs. echinoderms). However, a number of calcifying organisms have still managed to show either no change, or a positive response to elevated  $p\text{CO}_2$  conditions. For example, the brittlestar (*Amphiura filiformis* Müller, 1776) was capable of increasing their calcification rate by elevating their metabolic rate, but they also observed an energy deficit exhibited by substantial muscle wastage (Wood *et al.*, 2008). Other recent work found that mussels (*Mytilus edulis* L., 1758) in the Kiel Fjord (Germany) were capable of succeeding despite seasonal upwelling bringing  $\text{CO}_2$ -rich water at levels that exceed future predicted levels of  $p\text{CO}_2$  conditions (Thomsen *et al.*, 2010). Further investigation found that sufficient food availability was enough to compensate for the additional energetic costs of living, even when elevated  $p\text{CO}_2$  conditions were experimentally manipulated in addition to the  $\text{CO}_2$ -rich upwelling (Thomsen *et al.*, 2012).

In my opinion, these examples highlight important aspects that were subsequently incorporated into the experiments presented in this thesis. Firstly, that the effects of climate change need to be considered holistically for an organism, capturing both their ecological performance as well as physiological tolerance. For the brittlestar, had muscle wastage not been measured (or observed), then their performance in terms of calcification could have seen them labelled as a 'winner' in climate change, while the very opposite could be said to be true over the long-term. Secondly, the mussels experienced additional energetic costs of living which they were only able to overcome given sufficient food availability. Hence, the responses of marine organisms will not only be associated with their own sensitivity, it will also be tightly coupled with their ability to obtain energy, and certainly for heterotrophs, the sensitivity of those species that are trophically linked.

### 7.2.3 Trophic interactions - Energy acquisition

When considering the energy intake of an organism it is not just the availability of food that matters, food quality and the ability to capture and assimilate that food will all dictate the amount of energy that an organism can uptake. This is one of the principal findings in [Chapter 3](#). The prey resource (acorn barnacle, *Semibalanus balanoides*), demonstrated a reduced prey quality (reduced tissue production), but demonstrated no reduction in their shell mass. Thus, feeding by the predator (adult *Nucella lapillus*) resulted in a reduced reward for the same effort, suggesting that they would need to initiate compensatory feeding to maintain the same energy intake. However, in [Chapter 3](#), it was importantly found that long-term exposure to the reduced prey quality was sufficient enough to prevent them from being able to initiate compensatory feeding, and they subsequently exhibited tissue loss.

When *N. lapillus* were feeding upon a less sensitive prey resource *M. edulis* ([Chapter 4](#)), they were still negatively impacted by ocean acidification and warming, however, not to the same extent as those feeding on the barnacles, and the gastropods did not exhibit the same somatic tissue loss (i.e. starvation). Overall, this could represent a difference between life-stages ([Chapter 3](#) - adult, [Chapter 4](#), juvenile), but more likely suggests that the more tolerant mussel was able to provide a greater energetic content - although that was still not sufficient to completely compensate for the increased energetic costs in warmer, high CO<sub>2</sub> oceans. It is therefore anticipated that the consequences for predator-prey dynamics will be strongly dictated by the sensitivity of both predator and prey (see [Figure 7.1](#) for a conceptual figure).

In [Chapter 4](#), the biological complexity of the climate-change experiments was increased by incorporating a third trophic level, the shore crab *Carcinus maenas*, which acted as predator to our intermediate consumers, *N. lapillus* and *Littorina littorea*. Although consumption will have profound implications for the individual (i.e. death), the fitness of consumers can be influenced by non-consumptive effects of the predator. A key aspect of this is that the ability to avoid predators detracts from the energetic intake of the individual - i.e. the trade-off between eating and being eaten ([Trussell & Schmitz, 2012](#)). The findings of [Chapter 4](#), however, suggest that the effectiveness of these non-consumptive effects is highly dependent on the physiological state of the intermediate prey (i.e. *N. lapillus* and *L. littorea*), with the effect inversely proportional to reserves (i.e. towards being hunger-driven, rather than being balanced with the need for safety). Subsequently, the intermediate prey will likely need to become less risk-adverse and spend an increased amount of time foraging in a risky environment, in order for energy intake to match expenditure (i.e. the 'cost of living' - their metabolic rate).

Energy allocation strategies will typically prioritise those functions required for basal maintenance, allocating the remainder towards fitness-related activities, such as tissue production, calcification and reproduction ([Kooijman, 2010](#)). Based on ecological theory (dynamic energy budget, [Kooijman, 2010](#)), with feeding rates diminished, and a greater amount of energy used to meet overheads (somatic and maturity maintenance; [Figure 7.2](#)), the available energy that can be allocated towards reproduction is also likely to be reduced (see [Figure 7.2](#) for a conceptual image).

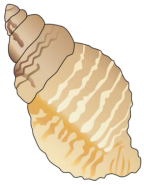





		Sensitive		Tolerant	
Predator					
Prey		Sensitive	Tolerant	Sensitive	Tolerant
					
Handling time		↓	= ↑	↓ ↓	=
Satiation		↓	=	↓	=
Compensatory feeding?		✓	✗	✓	✓
Predation rate		= ↑	=	↑ ↑	↑
Energy Budget		= ↓	↓	= ↑	=

Figure 7.1: Implications of differences in the sensitivity and tolerance of species influencing their predator-prey dynamics, including handling time, satiation, compensatory feeding, overall predation rate and implications for their resulting energy budget. Arrows indicate the whether the process is increased (↑), reduced (↓), or unchanged (=). Since the direction of processes can differ (e.g. reduced handling time, but increased predation rate is beneficial for an organism), the colour of the arrows indicates whether the predicted change is beneficial (green) or negative (red) for the organism. Compensatory feeding can either be initiated (✓) or not (✗).



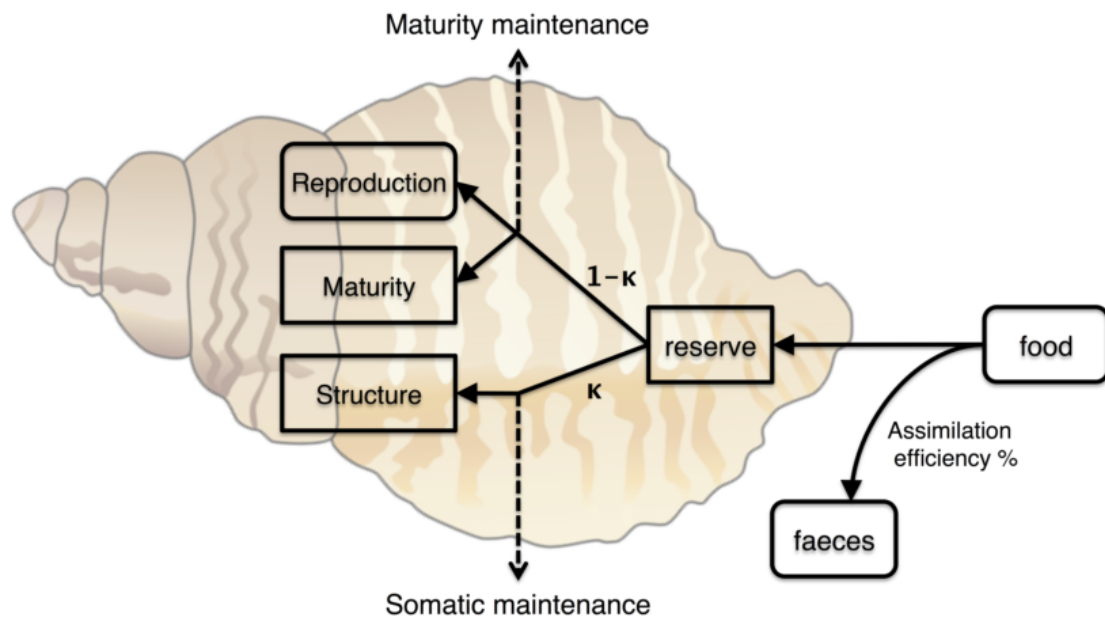


Figure 7.2: An example of dynamic energy budget theory. Food is ingested at a size-dependent rate (based on food density). Amount of energy extracted from the food is based on assimilation efficiency, and energy extracted is added to reserves. Somatic maintenance (i.e. overheads) are prioritised, and a fixed proportion of the available energy ( $\kappa$ ) is always used for growth of structure and maintenance. Afterwards, only the remaining energy ( $1 - \kappa$ ) is allocated to maturity (for embryos and juveniles) or reproduction (for adults). Solid lines indicate the allocation of resources, and dashed lines indicate the loss of energy due to overheads/maintenance.

#### 7.2.4 Population-level effects and energy allocation

The findings of [Chapter 3](#) and [Chapter 4](#) highlight shifts in the characteristics of consumer-resource dynamics, which importantly altered the response of *N. lapillus* and *L. littorea* to future climate change. In particular, both chapters found alterations in energy allocation strategies, with the intimation that less energy is likely to be allocated to reproduction and hence fitness (specifically, lifetime reproductive success) will be reduced. These responses cannot, however, always be directly translated into changes in distribution and abundance. This is because scaling up from the individual to population-level responses will require an appreciation of how climate change can not only influence the physiology of the individual, but also key demographic transitions that influence population dynamics (e.g. gene flow, population size).

Species with large population sizes and fast population turnover rates, such as phytoplankton, represent a model species for laboratory-based investigations into population-level responses to climate change, including experimental evolution (e.g. [Lohbeck et al., 2012](#)). These types of experiments, are however, likely to be carried out in the absence of more complex trophic and ecological interactions ([Harvey et al., 2014](#)). Moreover, these same experimental approaches are often not feasible for longer-lived species, which would likely need to be maintained under experimental conditions for months to years in order to achieve the necessary number of generations (but see, for example [Dupont et al., 2012](#); [Miller et al., 2012](#); [Parker et al., 2012](#); [Thor & Dupont, 2015](#)). Therefore, [Chapter 6](#) investigated the individual and population-level responses of the predatory gas-

tropod *Hexaplex trunculus* in response to long-term chronic exposure to elevated  $p\text{CO}_2$  conditions in the  $\text{CO}_2$  seep site in Vulcano (Sicily, Italy). This allowed experimental work to capture the individual physiological responses (including reproductive success) following multi-generational chronic exposure, whilst maintaining the trophic interactions that an organism experiences within a naturally-assembled community.

In [Chapter 6](#), it was found that elevated  $p\text{CO}_2$  conditions also increased the energetic demands of the individuals, which resulted in an altered energy allocation strategy (similar to [Chapter 3](#) and [Chapter 4](#)). This was demonstrated through elevated  $p\text{CO}_2$  conditions causing an increase in body size, and due to the increased body size, potentially increased gonad size. However, this also resulted in a simultaneous negative effect on the size, thickness and shape of the shell. For the individual, such changes in the size and thickness of the shell could result in an increased susceptibility to predation (Kroeker-2014), and influence their survival rates, and for the population, the (potentially) increased gonad size could enhance reproductive output. However, this does make the assumption that climate-driven stressors do not prevent individuals from achieving the advanced stages of gonad development (e.g. [Moore et al., 2011](#)) despite the gonads increasing in size.

When investigating the population-level, it was found that population size was reduced in the elevated  $p\text{CO}_2$  conditions, and the proportion of females was reduced (relative to the males). Moreover, based on the genetic signatures, it was found that not every individual contributed to reproduction, with different individuals contributing with each reproductive cycle (termed ‘increased variance in reproductive success’). Subsequently, in addition to the direct physiological responses, important demographic transitions will play a key role in determining the population-level consequences of future climate change. This includes whether sex-specific responses exist (e.g. [Vaughn et al., 2014](#)), how population size and density (including both effective population size, as well as the ability to find a mate) may alter, as well as the potential for adaptive evolution ([Reusch, 2013](#); [Sunday et al., 2014](#)).

Adaptive evolution may represent a critical mechanism which could alleviate some of the negative consequences expected with future climate change ([Gienapp et al., 2008](#); [Harvey et al., 2014](#)). However, the increased variance in reproductive success that was observed would lead to greater levels of short-term genetic drift, which is predicted to oppose adaptation. Although the  $\text{CO}_2$  seeps are not a perfect analogue for future climate change (e.g. single stressor), this highlights that while some species may be capable of achieving adaptive evolution (e.g. [Lohbeck et al., 2012](#); [Pespeni et al., 2013a,b](#)), environmental influences on the contemporary demographics of some species may represent a countervailing mechanism against adaptation (e.g. [Conover & Present, 1990](#); [Marcil et al., 2006](#)). Therefore, despite high gene flow being maintained across the study site in [Chapter 6](#), ocean acidification still demonstrated important individual and population-level changes, that will likely translate to profound shifts in the ecosystem.

### Community restructuring and ecosystem function

Since it is recognised that some species are more tolerant than others ([Chapter 2](#)), the challenge is to understand how such differences will affect ecosystems ([Dupont & Pörtner, 2013](#)). Given the general consensus that a positive relationship exists between biodiversity and ecosystem functioning ([Hooper et al., 2005](#); [Balvanera et al., 2006](#); [Cardinale](#)

*et al.*, 2006; Hooper *et al.*, 2012; Gamfeldt *et al.*, 2015), there is now a pressing need to understand how changes in biodiversity and altered community structure (i.e. loss of sensitive species) will influence the goods and services that humans derive from ecosystems (e.g. Alsterberg *et al.*, 2013; Eklöf *et al.*, 2015).

In Chapter 5, the effects of ocean acidification, warming and the diversity of grazing assemblages were tested on the ecosystem functioning of a microphytobenthos (MPB) community. It was importantly demonstrated that biodiversity is important for ecosystem functioning, regardless of the environmental conditions. However, ecosystems do not simply possess a single function or process, instead they possess multi-functionality (Byrnes *et al.*, 2014). Hence, the key finding of Chapter 5 was that, in general, increased biodiversity appeared to influence several ecosystem processes (consumer net effect/resource utilisation, gross primary production, and standing stock) and hence, either promote or at least sustain ecosystem multi-functionality (i.e. the positive functioning of the ecosystem, across several ecosystem processes). However, the ability to sustain multi-functionality at the elevated temperatures resulted in a trade-off between ecosystem processes, meaning that it was no longer possible to sustain all the processes. This was predominantly associated with increased temperature causing a shift towards heterotrophy (also see Lopez-Urrutia *et al.*, 2006; O'Connor *et al.*, 2009; Hicks *et al.*, 2011), reducing the standing stock and production of the MPB communities.

Another interesting observation was that the potential for increased  $p\text{CO}_2$  to be used as a resource by the autotrophs (e.g. Connell *et al.*, 2013) was limited by enhanced top-down control, or trophic compensation (Ghedini *et al.*, 2015). Thus, it seemed that the community-level effects associated with warming and altered biodiversity outweighed those of ocean acidification. A similar finding was found in a study of a seagrass-associated community (Eklöf *et al.*, 2015), and highlights that it may be possible to find generalisations between ecosystem types, and that these sorts of responses should be tested further to search for unifying principles.

The diversity effects observed here appeared to be strongly driven by identity effects (i.e. specific traits of the grazer promoted different ecosystem processes), rather than a general species effect *per se*, and hence did not suggest much within-process functional redundancy between the three species (Reiss *et al.*, 2009; Perkins *et al.*, 2010a). As such, any species loss would certainly have a negative impact on ecosystem processes described here. A number of recent studies have highlighted the need for high levels of biodiversity to maintain multifunctionality (e.g. Gamfeldt *et al.*, 2008; Isbell *et al.*, 2011; Maestre *et al.*, 2012a,b). Clearly, the levels of biodiversity manipulated in this experiment were low relative to natural systems, and so it may be possible for our MPB and grazer assemblage to sustain all processes (i.e. not require the trade-offs) - if the biodiversity were increased (Gamfeldt *et al.*, 2008; Perkins *et al.*, 2015). However, it has also been found that even higher biodiversity may be required to sustain ecosystem multifunctionality with increasing environmental change (e.g. Perkins *et al.*, 2015).

### 7.3 FUTURE RESEARCH DIRECTIONS

As demonstrated by this thesis, the effects of future ocean acidification and warming are likely to have individual-, population-, community- and ecosystem-level consequences, with further interactions between these levels of biological hierarchy. Consequently, the

future direction for research needs to design experiments that can embrace this complexity, but still aim to establish generalisations that are applicable across different ecosystem types.

The first step in moving beyond single species towards and understanding of community and ecosystem-level responses will require identifying which species should be studied. The choice of species could be associated with the needs for either ecosystem's services or functioning, such as the role of coccolithophores in carbon cycling, or possibly in an ecological context, being a habitat forming or keystone species. Future research should then be directed towards the chosen species, but also include their interactions with co-existing species. Moreover, given the different sensitivities of each life-stage, responses will need to be considered trans-generationally in order to capture the response for all ontogeny, life-stages and across generations. This could be achieved through the use of chronic long-term multigenerational experiments and/or natural analogues.

Clearly, these aspects of future research cannot all be achieved in a single experiment. Instead, establishing unifying theories will require investigating biological responses both spatially and temporally, by utilising spatially representative replication across different scientific disciplines and research institutes. It is hoped that this should contribute towards integrating responses at regional or global scales, allowing direct comparisons, and provide a more integrative picture of the responses at the community and ecosystem levels.

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## Part II

## APPENDICES





SUPPORTING INFORMATION FOR CHAPTER 2

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## Supporting Information for Chapter 2

Supporting information for [Chapter 2](#) can be found archived online with the published manuscript:

Ben P. Harvey, Dylan Gwynn-Jones and Pippa J. Moore (2013) Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecology and Evolution*, 4: 1016-1030.

Supporting information archived at:

<http://onlinelibrary.wiley.com/doi/10.1002/ece3.516/supinfo>

## Supporting information captions

Table A.1: Experiments included in meta-analysis. Each row represents an individual experiment that was included for meta-analysis. Columns '2-6' describes the experiment as: the manipulated stressor - elevated  $p\text{CO}_2$  (OA), temperature (T), taxonomic group (Crustose Coralline Algae - CCA), species, trophic level and life-stage. Columns '7 - 11' describe the number of times each response (Calcification, growth, photosynthesis, reproduction and survival) was tested.

Table A.2: Selection criteria for exclusion in meta-analysis. Each row represents an individual observation that was omitted from subsequent analysis. Therefore, some studies may include a number of observations, in which some are included (and listed within ST1) and some are omitted. Columns '2 - 6' describes the experiment as: the manipulated stressor, response, taxonomic group, species and life-stage. Columns '7 - 12' describe the reason that particular experiment did not meet the criteria. Stressor Level describes when either the  $\text{CO}_2/\text{pH}$  or temperature manipulation was greater than the IPCC 2100 predictions (i.e.  $>0.5$  pH reduction,  $>1300\text{ppm}$   $\text{CO}_2$ , or  $>5$  °C increase). Response indicates that the particular response variable of that experiment did not have a sufficient number to be quantitatively assessed. Fieldwork indicates that the experiment was carried out in the field and therefore omitted because of possible confounding factors. No Variance highlights that either the study did not provide a form of uncertainty (either standard deviation, standard error or confidence interval) or that the study only had 1 replicate. Carbonate Chemistry indicates that the carbonate chemistry of the experiment was manipulated using an HCL Addition rather than manipulating the DIC. Other reason highlights a reason that the experiment was omitted that did not fall into one of the preceding categories. Any experiment that did not meet a particular criteria was omitted, and reasons for omission were not investigated further. As such, the criteria is not definitive and any experiment on the list may fail to meet additional criteria.

Table A.3: Heterogeneity Tests - Within Groups (Q) and Between Groups ( $Q_M$ ). Heterogeneity statistics for each model in the different response variables. Separate analyses were conducted to compare similarity in effect size between each group. Q - Heterogeneity,  $Q_M$  - Heterogeneity explained by differences between subgroups,  $Q_E$  - Residual Heterogeneity. \*\*\*'  $<0.001$ , '\*\*'  $<0.01$ , '\*'  $<0.05$ , '.'  $<0.1$ .



SUPPORTING INFORMATION FOR CHAPTER 3

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**Supporting information for Chapter 3** Akaike information criterion (AIC) was used to compare linear and non-linear ( $a \cdot \text{RCD}^b$ ) regressions. In most cases, non-linear regressions were deemed more appropriate for both RCD to dry mass and RCD to shell mass (AIC values are provided below).

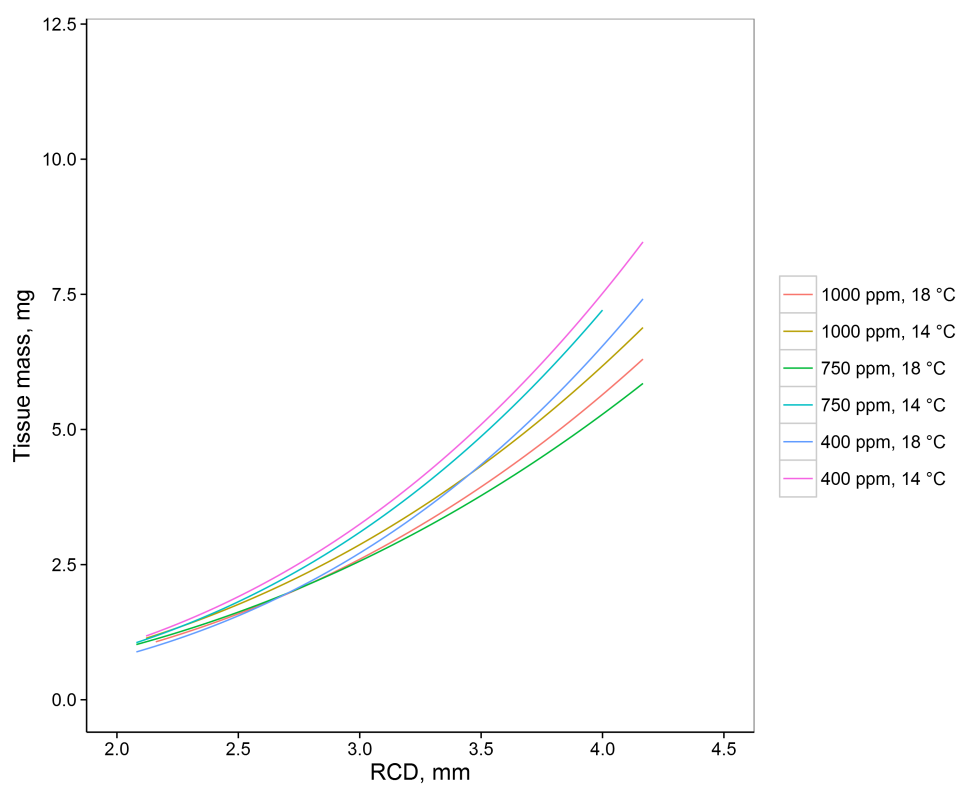


Figure B.1: Allometric relationship between tissue mass (mg) and rostro-carinal diameter (RCD, mm) in response to ocean acidification (400, 750 and 1000ppm) and temperature (14 and 18 °C).

Table B.1: Akaike information criterion (AIC) values for the linear ( $A \times \text{RCD} + B$ ) and allometric ( $A \times \text{RCD}^B$ ) models to describe the relationship between the tissue mass and rostro-carinal diameter (RCD) of *S. balanoides*.

AIC	Linear ( $A \times \text{RCD} + B$ )	Allometric ( $A \times \text{RCD}^B$ )
1000ppm, 18 °C	237.46	229.84
1000ppm, 14 °C	268.97	267.33
750ppm, 18 °C	229.14	235.97
750ppm, 14 °C	325.67	316.36
400ppm, 18 °C	300.11	284.54
400ppm, 14 °C	342.49	334.61

Table B.2: Summary of two-way ANCOVA of (log) tissue mass in response to ocean acidification and warming, with (log) length as a covariate.

	Estimate	Std. Error	t value	Pr(> t )	
(Intercept)	-0.74	0.047	-15.71	< 2e-16	***
Length	2.819	0.057	49.08	< 2e-16	***
pCO <sub>2</sub>	-0.037	0.018	-2.04	0.042	*
Temperature	-0.087	0.025	-3.53	0.0005	***
pCO <sub>2</sub> :Temperature	0.012	0.011	1.05	0.293	

Table B.3: Matrix of *p*-values from the pairwise tests (Tukey HSD) of the ANCOVA of (log) tissue mass in response to ocean acidification and warming, with (log) length as a covariate. Results are mirrored to aid interpretation.

	1000ppm, 18 °C	1000ppm, 14 °C	750ppm, 18 °C	750ppm, 14 °C	400ppm, 18 °C	400ppm, 14 °C
1000ppm, 18 °C	-	0.245	0.659	<0.001 ***	0.607	<0.001 ***
1000ppm, 14 °C	0.245	-	0.003 **	0.250	0.991	0.026 *
750ppm, 18 °C	0.659	0.003 **	-	<0.001 ***	0.025 *	<0.001 ***
750ppm, 14 °C	<0.001 ***	0.250	<0.001 ***	-	0.062 .	0.945
400ppm, 18 °C	0.607	0.991	0.025 *	0.062 .	-	0.003 **
400ppm, 14 °C	<0.001 ***	0.026 *	<0.001 ***	0.945	0.003 **	-



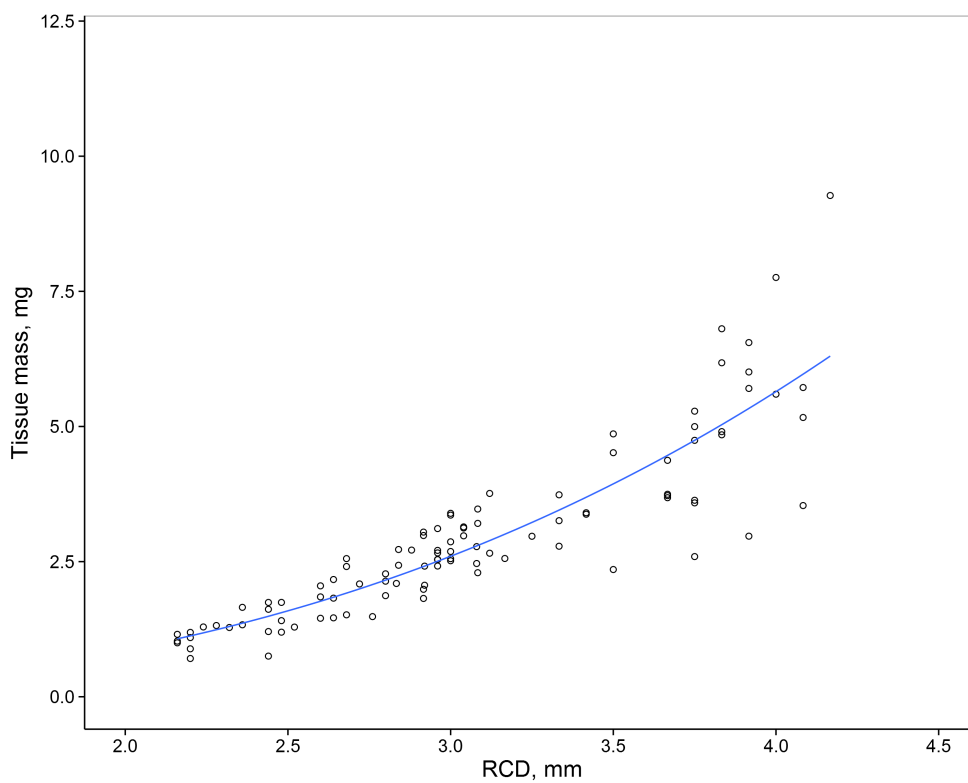


Figure B.2: Allometric equation between rostro-carinal diameter (RCD) and tissue mass (mg) at 1000 ppm and 18 °C. Tissue mass (mg) =  $0.1344 * \text{RCD (mm)}^{2.6963}$ ,  $n = 100$ .

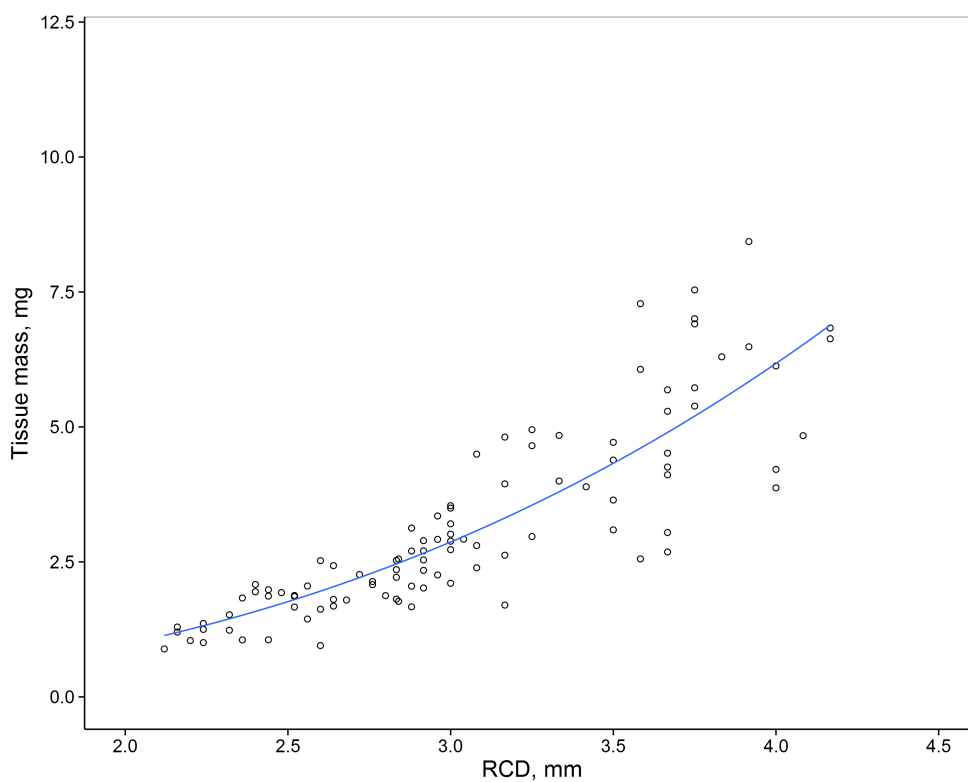


Figure B.3: Allometric equation between rostro-carinal diameter (RCD) and tissue mass (mg) at 1000 ppm and 14 °C. Tissue mass (mg) =  $0.1534 * \text{RCD (mm)}^{2.6654}$ ,  $n = 100$ .

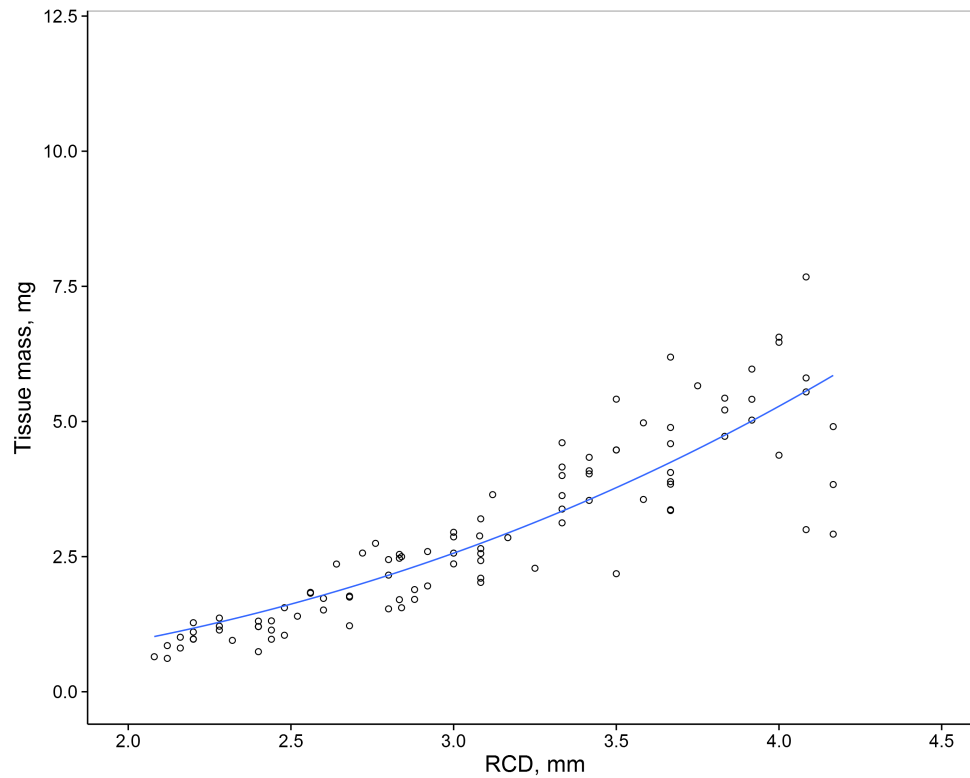


Figure B.4: Allometric equation between rostro-carinal diameter (RCD) and tissue mass (mg) at 750 ppm and 18 °C. Tissue mass (mg) =  $0.1622 * \text{RCD (mm)}^{2.5126}$ ,  $n = 100$ .

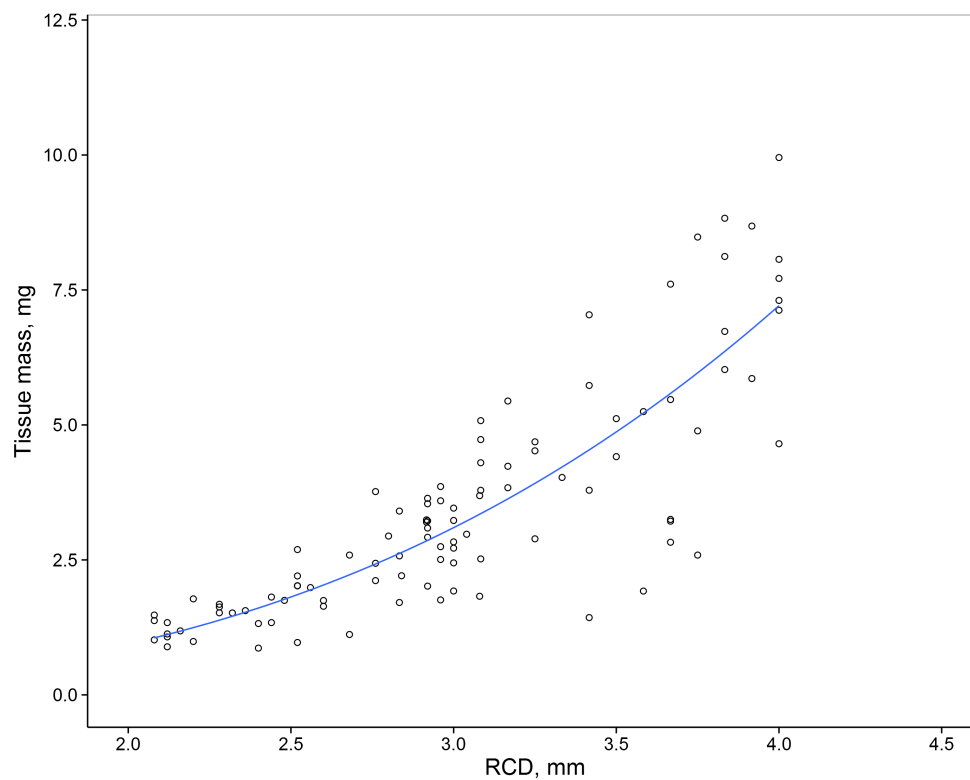


Figure B.5: Allometric equation between rostro-carinal diameter (RCD) and tissue mass (mg) at 750 ppm and 14 °C. Tissue mass (mg) =  $0.1230 * \text{RCD (mm)}^{2.9363}$ ,  $n = 100$ .

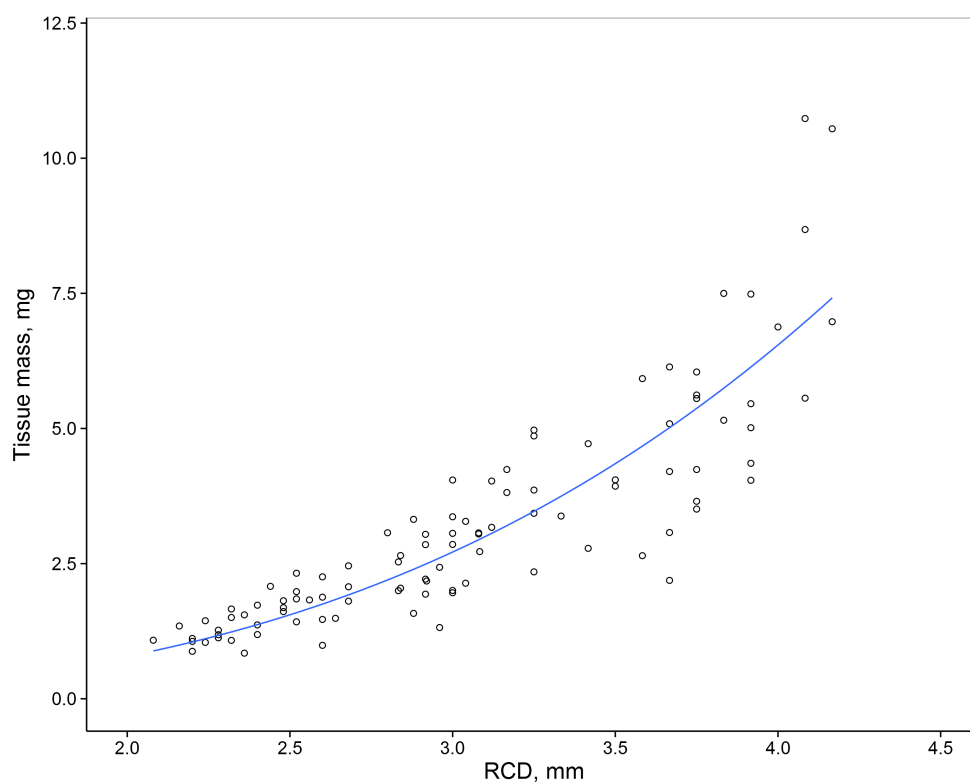


Figure B.6: Allometric equation between rostro-carinal diameter (RCD) and tissue mass (mg) at 400 ppm and 18 °C. Tissue mass (mg) =  $0.0941 * \text{RCD (mm)}^{3.0601}$ ,  $n = 100$ .

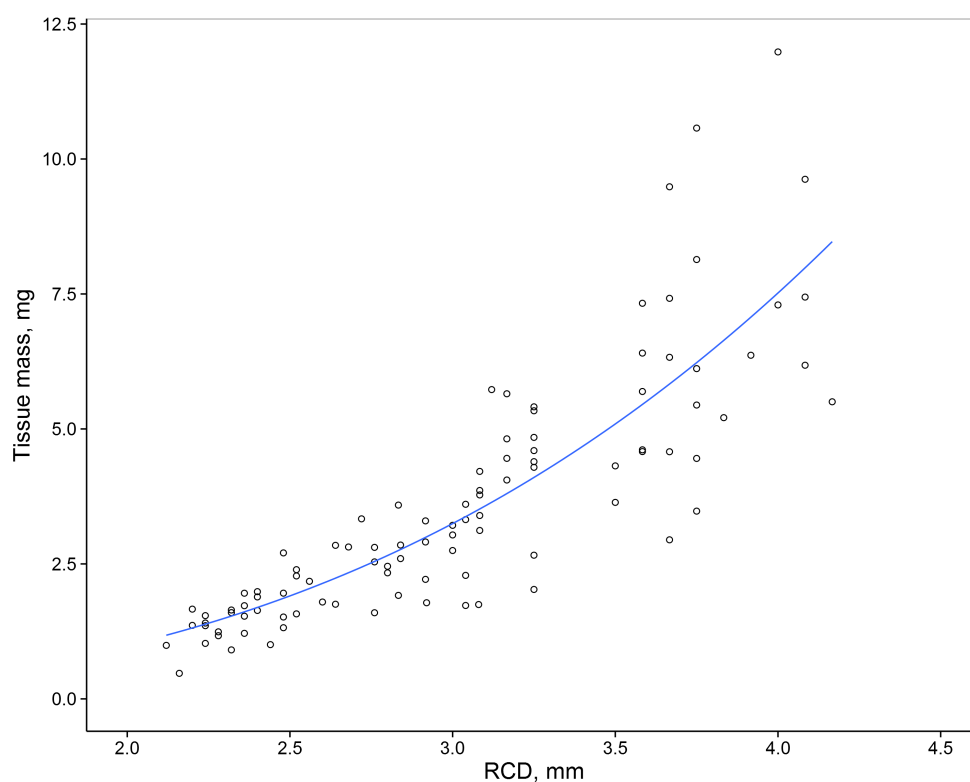


Figure B.7: Allometric equation between rostro-carinal diameter (RCD) and tissue mass (mg) at 400 ppm and 14 °C. Tissue mass (mg) =  $0.1117 * \text{RCD (mm)}^{3.0622}$ ,  $n = 100$ .

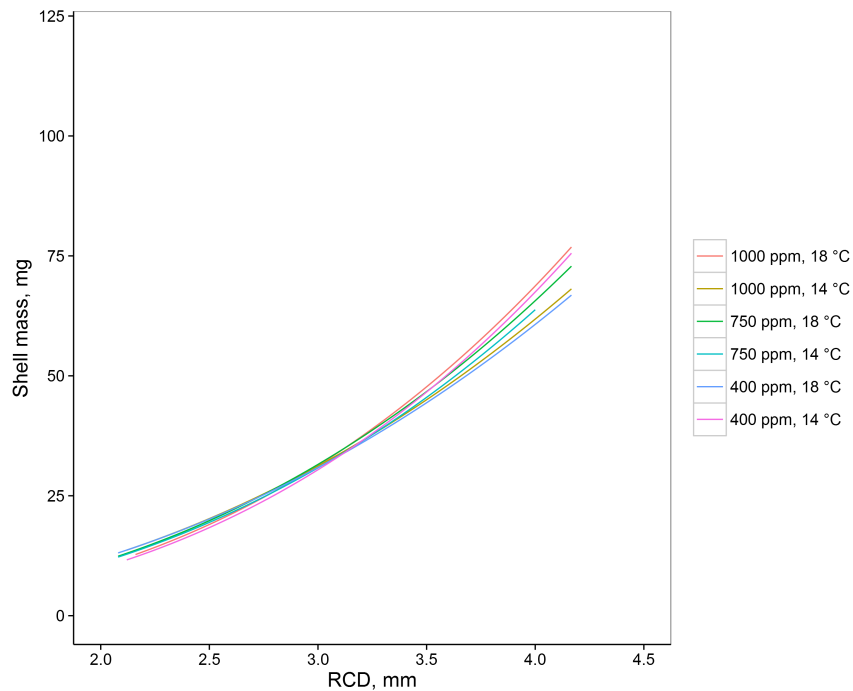


Figure B.8: Allometric relationship between shell mass (mg) and rostro-carinal diameter (RCD, mm) in response to ocean acidification (400, 750 and 1000ppm) and temperature (14 and 18 °C).

Table B.4: Akaike information criterion (AIC) values for the linear ( $A \times \text{RCD} + B$ ) and allometric ( $A \times \text{RCD}^B$ ) models to describe the relationship between the shell mass and rostro-carinal diameter (RCD) of *S. balanoides*. AIC is a measure of the relative quality of a statistical model for a given set of data, with numbers closer to zero deemed a more appropriate model.

AIC	Linear ( $A \times \text{RCD} + B$ )	Allometric ( $A \times \text{RCD}^B$ )
1000ppm, 18 °C	740.97	728.73
1000ppm, 14 °C	808.75	809.34
750ppm, 18 °C	745.24	740.68
750ppm, 14 °C	838.88	838.84
400ppm, 18 °C	798	798.55
400ppm, 14 °C	843.19	839.07

Table B.5: Summary of two-way ANCOVA of (log) shell mass in response to ocean acidification and warming, with (log) length as a covariate.

	Estimate	Std. Error	<i>t</i> value	Pr(>  <i>t</i>  )	
(Intercept)	0.358	0.055	6.518	1.57E-10	***
Length	0.355	0.01	35.378	< 2e-16	***
pCO <sub>2</sub>	0.0007	0.021	0.033	0.974	
Temperature	0.005	0.029	0.183	0.855	
pCO <sub>2</sub> :Temperature	0.007	0.013	0.491	0.624	

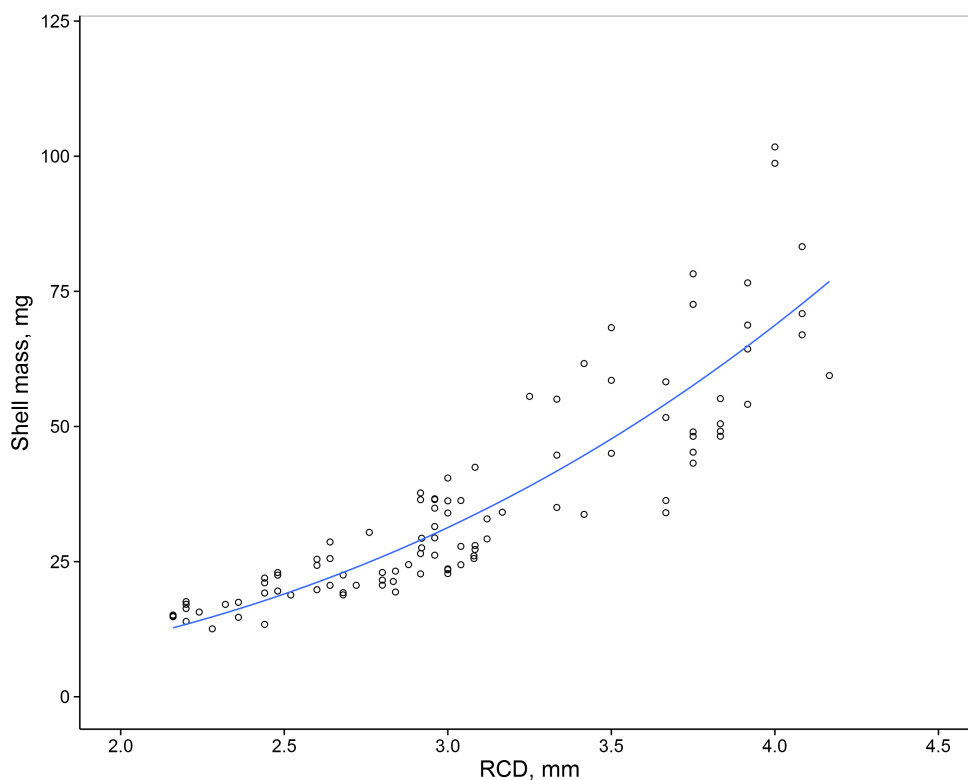


Figure B.9: Allometric equation between rostro-carinal diameter (RCD) and shell mass (mg) at 1000 ppm and 18 °C. Shell mass (mg) =  $1.5532 * \text{RCD (mm)}^{2.7338}$ ,  $n = 100$ .

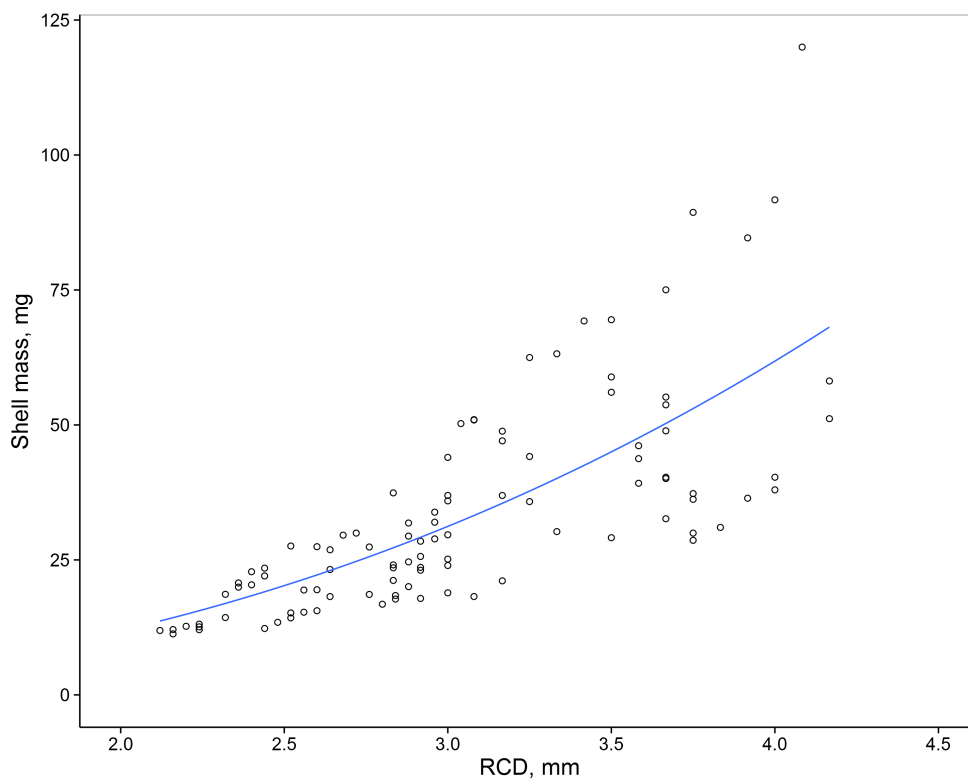


Figure B.10: Allometric equation between rostro-carinal diameter (RCD) and shell mass (mg) at 1000 ppm and 14 °C. Shell mass (mg) =  $2.2939 * \text{RCD (mm)}^{2.3761}$ ,  $n = 100$ .

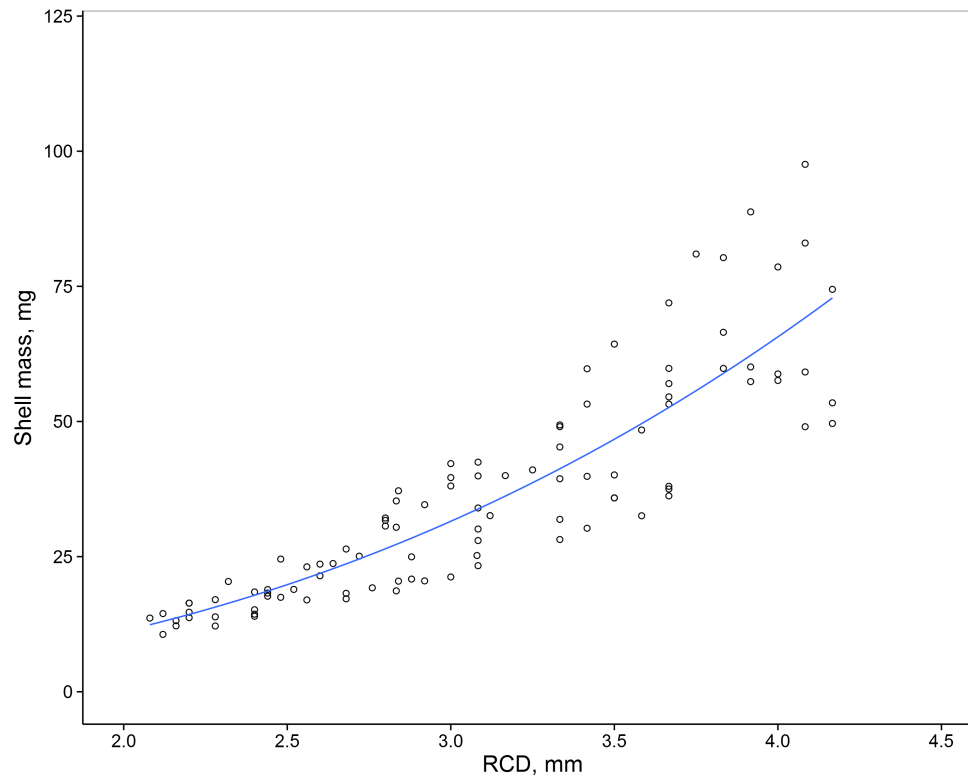


Figure B.11: Allometric equation between rostro-carinal diameter (RCD) and shell mass (mg) at 750 ppm and 18 °C. Shell mass (mg) =  $1.9167 * \text{RCD (mm)}^{2.5491}$ ,  $n = 100$ .

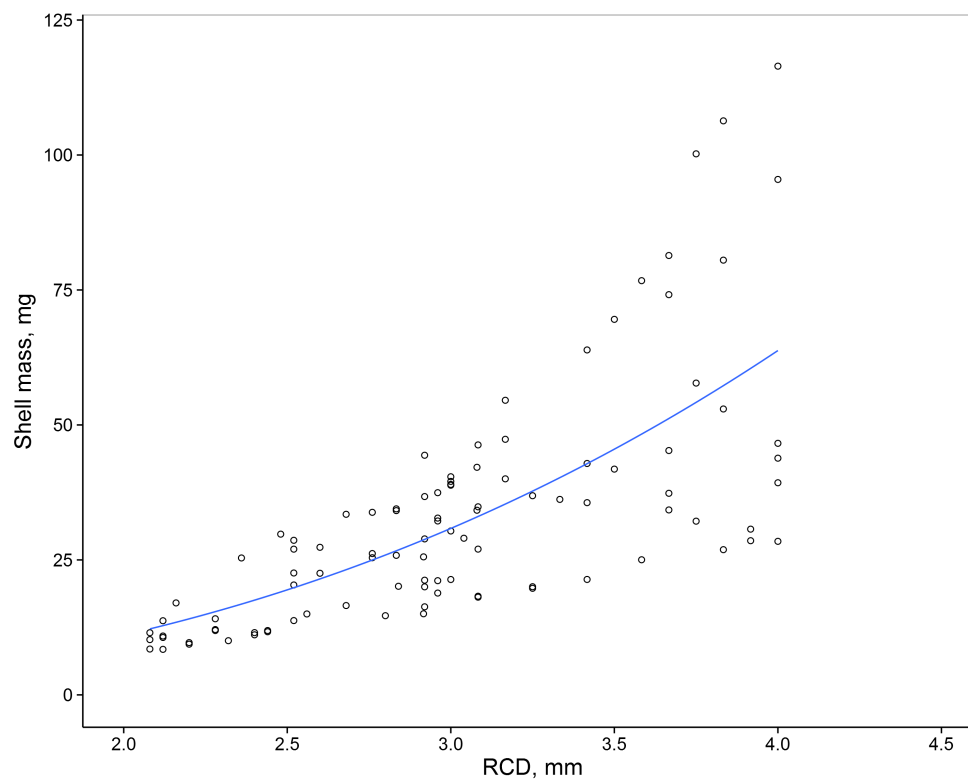


Figure B.12: Allometric equation between rostro-carinal diameter (RCD) and shell mass (mg) at 750 ppm and 14 °C. Shell mass (mg) =  $1.9210 * \text{RCD (mm)}^{2.5266}$ ,  $n = 100$ .

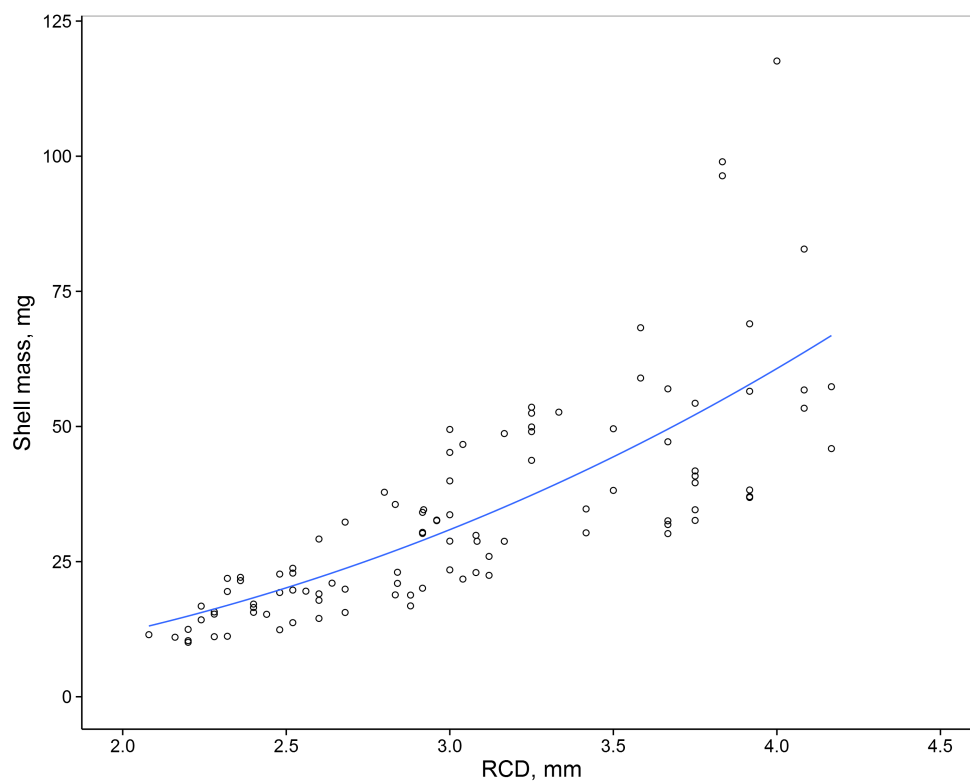


Figure B.13: Allometric equation between rostro-carinal diameter (RCD) and shell mass (mg) at 400 ppm and 18 °C.  $T$  Shell mass (mg) =  $2.3467 * RCD (mm)^{2.3467}$ ,  $n = 100$ .

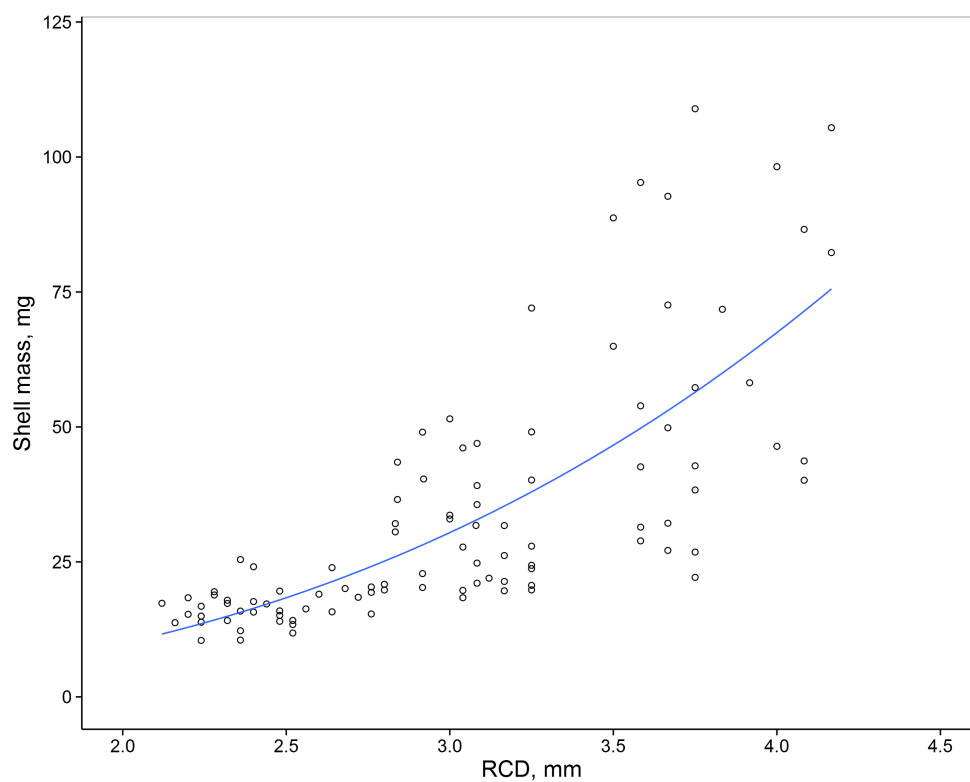


Figure B.14: Allometric equation between rostro-carinal diameter (RCD) and shell mass (mg) at 400 ppm and 14 °C. Shell mass (mg) =  $1.4529 * RCD (mm)^{2.7687}$ ,  $n = 100$ .





## SUPPORTING INFORMATION FOR CHAPTER 4

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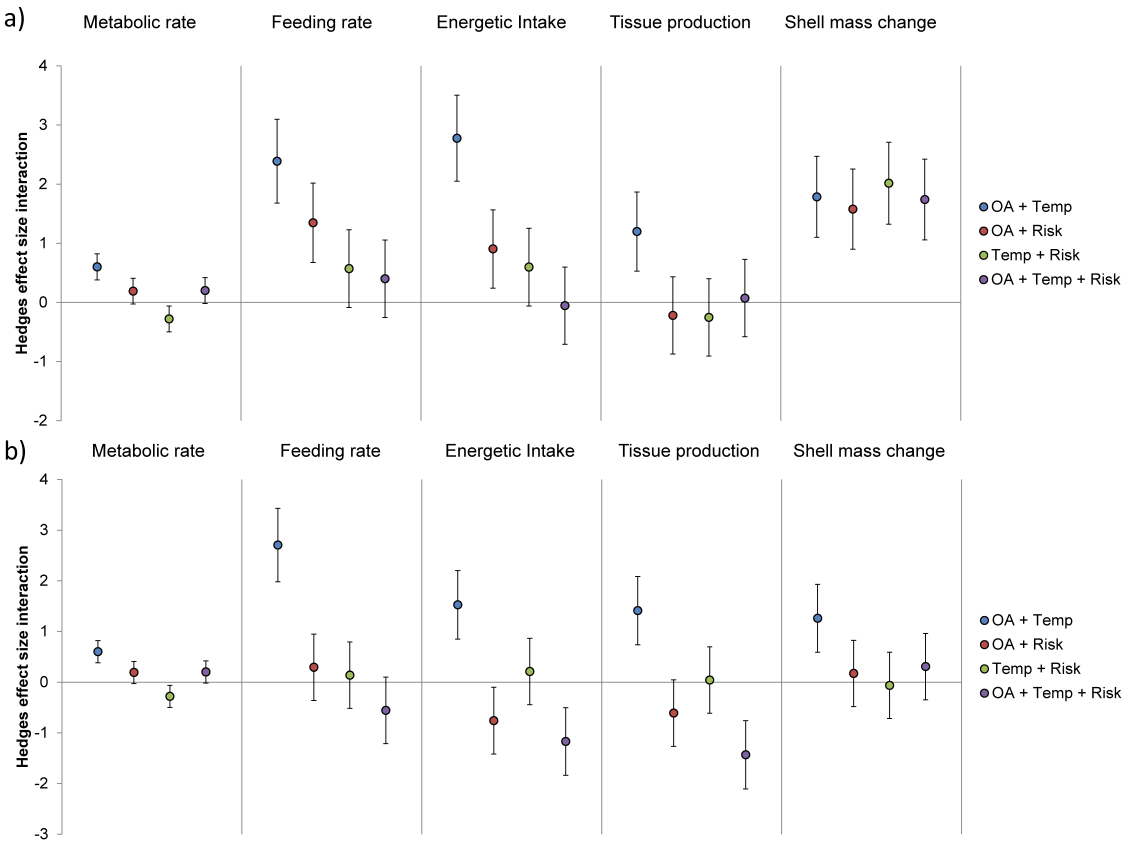


Figure C.1: Mean ( $\pm$  95% CI) Interaction between the effect sizes for metabolic rate, feeding rate, energetic intake, tissue production and shell mass change of a) *Nucella lapillus* and b) *Littorina littorea* in response to (i) elevated  $p\text{CO}_2$  and increased temperature (OA + Temp, blue), (ii) elevated  $p\text{CO}_2$  and predation risk (OA + Risk, red), (iii) increased temperature and predation risk (Temp + Risk, green) and (iv) combined elevated  $p\text{CO}_2$  and increased temperature, with predation risk (OA + Temp + Risk, purple). Positive values where the error bar does not cross zero are synergistic, Negative values where the error bars does not cross zero are antagonistic, and values where the error bar crosses zero are additive.

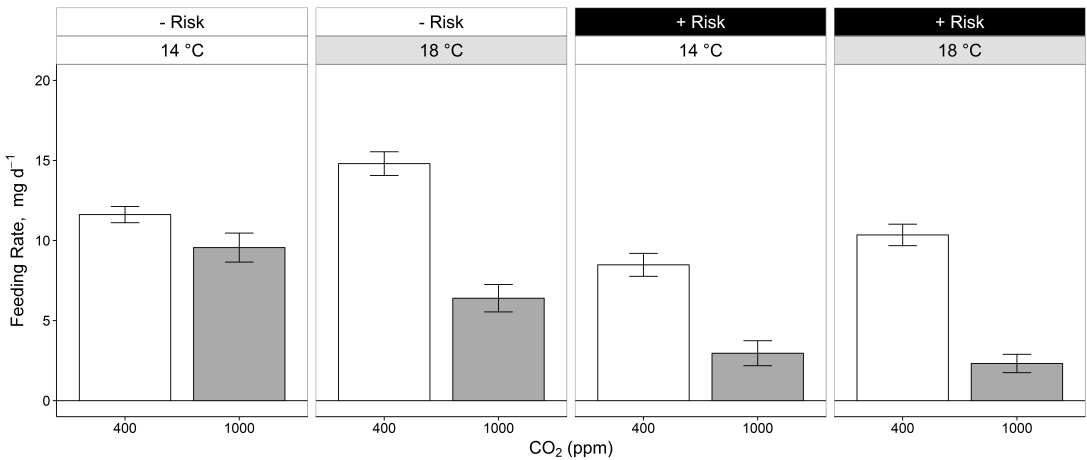


Figure C.2: Mean ( $\pm$  S.E) feeding rate ( $\text{mg d}^{-1}$ ) of *N. lapillus*,  $n = 6$ . See Table [Table 4.3](#) for significant differences.



SUPPORTING INFORMATION FOR CHAPTER 5

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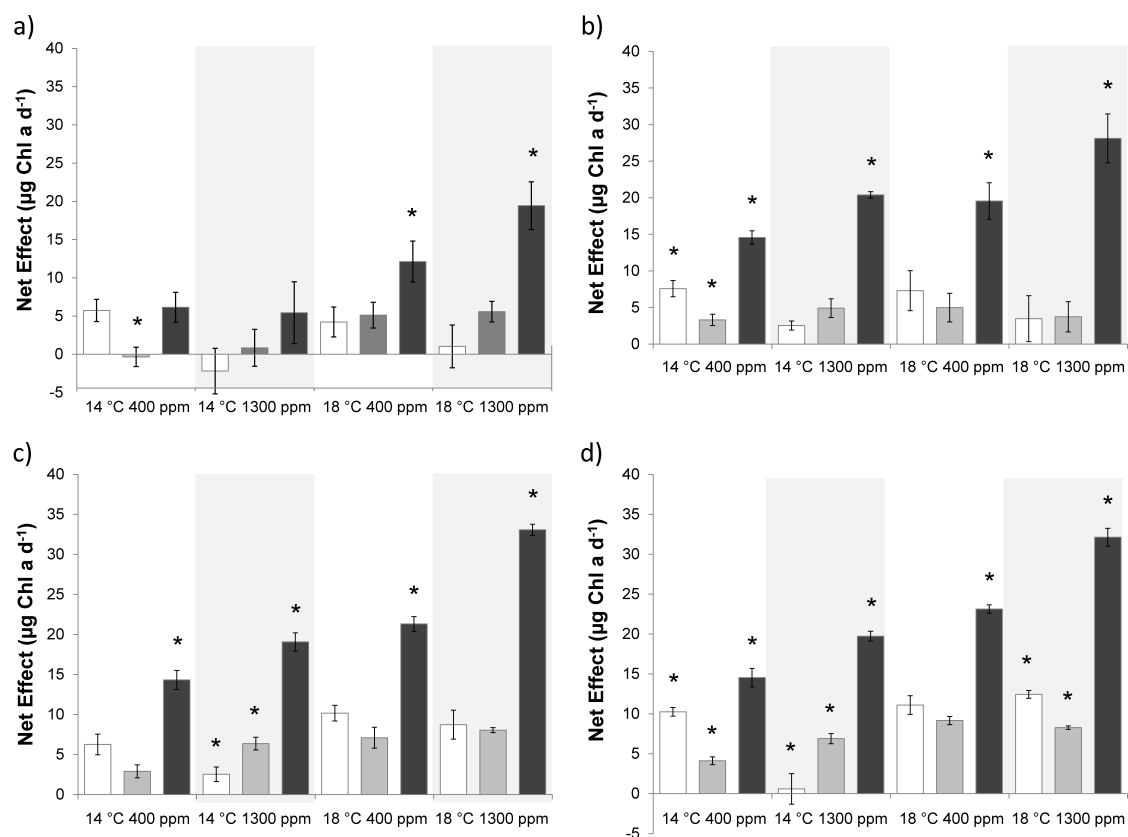


Figure D.1: Mean ( $\pm$  S.E) net effect ( $\mu\text{g Chl a d}^{-1}$ ) of a) the *P. vulgata* monoculture, b) the *G. umbilicalis* monoculture, c) the *L. littorea* monoculture, and d) the polyculture community, on the total MPB of three substrates - Barnacles (open bars), Hummocks (solid light grey bars) and Tile (solid dark grey bars). Statistical differences between substrate types (only) are indicated by an asterisk, with the asterisk indicating that a particular substrate type is significant from the other two types.

## PUBLISHED PEER-REVIEWED ARTICLES

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